



Contents

Original Articles

- Aas K. Hyposensitization in House Dust Allergy
Asthma: A Double blind Controlled Study with Evaluation of the Effect on Bronchial Sensitivity to House Dust 764
- Aperia, A. Berg U. and Broberger O. Control of Sodium Homeostasis in Children with Recurrent Urinary Tract Infections and Reduced Glomerular Filtration Rates 695
- Armata J. Wyszowski J. and Cyklic R. Rubidomycin in Blastoid Phases of Acute Promyelocytic and Myeloblastic Leukemia in Children 471
- Ashkenazi A. Yarora R. Gutman A. Abrahamov A. and Russell A. Niemann Pick Disease and Giant Cell Transformation of the Liver 285
- Barclay N. High Frequency of *Salmonella* Species as a Cause of Neonatal Meningitis in Ibadan Nigeria: A Review of Thirty-eight Cases 540
- Bartsocas C. S. Grunt J. A. Boylen Jr. G. W. and Brandt I. K. Oral D. Penicillamine and Intramuscular Bal. Edix in the Treatment of Lead Accumulation 553
- Berg, K. and Celander O. Circulatory Adaptation in the Thermoregulation of Fullterm and Premature Newborn Infants 278
- Berg U. Aperia A. and Broberger O. Subclinical Defects in Renal Regulation of Acid Base Balance in Children with Recurrent Urinary Tract Infections 521
- Berg U. Renal Function Tests in Nonacute Recurrent Urinary Tract Infections in Children 669
- Bergman L. and Isaksson B. Plasma Calcium Fractions in Normal Subjects from Birth to Adult Ages 630
- Bjerring Hansen A. Short Term Differences of Infant Development in Nursery Homes and in Private Families 571
- Bodegård G. and Schwieler G. H. Control of Respiration in Newborn Babies II The Development of the Thoracic Reflex Response to an Added Respiratory Load 181
- Bucci G. Mendicino M. Scalamandrè A. Annibaldi L. Savignoni P. G. and Nodari S. A Controlled Trial on Therapy for Newborns Weighing 750-1250 g. II Blood Chemistry and Electrocardiographic Observations in the Newborn Period 417
- Casteels van Daele M. and De Gaetano G. Purpura and Acetylsalicylic Acid Therapy 03
- Churga F. and Lardinois R. Separation by Gel Filtration and Microdetermination of Unbound Bilirubin I In Vitro Albumin and Acidosis Effects on Albumin Bilirubin Binding 27
- Cocchi P. Mori S. and Becattini A. Nitroblue Tetrazolium Reduction by Neutrophils of Newborn Infants in Vitro Phagocytosis Test 475
- Damgaard Andersen, L. Krasidnikoff P. A. and Overvad H. Intermittent Muscular Weakness Extrasystoles and Multiple Developmental Anomalies: A New Syndrome? 559
- Dar H. Carney Jr. F. E. and Winter S. T. Dermato-glyphics and the Simian Crease in Infants of Low Birth Weight: A Pilot Study 479
- Donath A. The Simultaneous Determination in Children of Glomerular Filtration Rate and Effective Renal Plasma Flow by the Single Injection Clearance Technique 512
- Ehrespreis T. Gierup J. and Lagercrantz, R. Chronic Regional Enterocolitis (Mb Crohn) in Children and Adolescents 209
- Lid E. E. A Follow Up Study of Physical Growth Following Failure to Thrive with Special Reference to a Critical Period in the First Year of Life 39
- Fernandes J. and van de Kamer J. H. The Combined Xylose Disaccharide Tolerance Test: Its Application for Diagnosing Disaccharidase Deficiency 187
- Finnstrom O. Studies on Maturity in Newborn Infants I Birth Weight, Crown Head Length, Head Circumference and Skull Diameters in Relation to Gestational Age 685
- Flood N. E. and Ackerman D. Perinatal Asphyxia and Residual Placental Blood Volume 431
- Foukard T. Berg, T. Johansson S. G. O. and Wahren B. Virus Serology and Serum IgE Levels in Children with Asthmatoïd Bronchitis 621
- Gottlieb A. Nir I. and Pesach J. Urinary Excretion of Free and Conjugated Glucuronic Acid in Jaundiced Newborn 437
- Grotte G. Olsen L. and Reuterskiöld A. Reconstruction of Oesophagus with Colonic Transposition 39
- Gustavson K. H. and Hagberg, B. The Incidence and Genetics of Metachromatic Leucodystrophy in Northern Sweden 585
- Haahr J. and Sparrevojn S. Epididymitis in Children: A Brief Review together with Reports of Six Cases 216
- Hjalmarsson, O. Jagenburg R. and Rodger S. Mild and Severe PKU: Comparative Studies in Two Infants 11
- Ilingsworth R. S. and Eid E. E. The Head Circumference in Infants and Other Measurements to which it may be Related 333
- Kanawati A. McLaren D. S. and Abu Jawdeh I. Failure to Thrive in Lebanon: I Experience with some Simple Somatic Measurements 309
- Kantzyk D. Klein N. Prinzheim W. and Kunzer W. Fibrinogen Turnover in the Premature Infant with and without Idiopathic Respiratory Distress Syndrome 465
- Kildeberg P. and Engel K. Metabolic Alkalosis in Infants: Role of Water Depletion and Changes in Composition of Stool: Review of a Physiological Problem 637
- Kintzel H. W. Hinkel G. K. and Schwarze R. The Decrease in the Serum Bilirubin Level in Premature Infants by Orotic Acid 1

- Kletter B Freier S Davies A M and Gery I The Significance of Coproantibodies to Cow's Milk Proteins 173
- Laskownicka Z Pasyk K Porebska A and Zem bura K Pimaricin (Natamycin) in the Treatment of Superficial Fungal Infections in Children 456
- Lie S O Loken A C Stromme J H and Aagaenae U Fetal Congenital Lactic Acidosis in Two Siblings I Clinical and Pathological Findings 129
- Lindstedt E Lindgård B and Lindholm T Arteriovenous Fistula for Haemodialysis in Children 78
- Lommen E J P Vogels G D van der Zee S P M Trybels J M F and Schretlen E D A M Concentration of Purine Nucleotides in Erythrocytes of Patients with the Lesch Nyhan Syndrome before and during Oral Administration of Adenine 642
- Lundström N R and Edler I Ultrasoundcardiography in Infants and Children 117
- Lyon I C T Procopis P G and Turner B Cystathioninuria in a Well Baby Population 324
- Margret W and Adam D Bay B 5097 A New Orally Applicable Antifungal Substance with Broad Spectrum Activity Preliminary Clinical and Laboratory Experiences in Children 341
- Matoth Y Zaizov R and Varsano I Postnatal Changes in Some Red Cell Parameters 317
- Mendicino M Scalapandré A Savignoni P G Picece Bucci S Esuperanzi R and Bucci G A Controlled Trial on Therapy for Newborns Weighing 750-1250 g I Clinical Findings and Mortality in the Newborn Period 407
- Nordio S Donath A Macagno F and Gatti R Chronic Hypomagnesemia with Magnesium Dependent Hypocalcemia I A New Syndrome with Intestinal Magnesium Malabsorption 441
- Nordio S Donath A Macagno F and Gatti R Chronic Hypomagnesemia with Magnesium Dependent Hypocalcemia II A Study of the Relationship between Magnesium Calcium and Strontium 449
- Norén J Carlsson E Kretzschmar G and Teger Nilsson A C Prothrombin in Newborns and during the First Year of Life 269
- Norman A Strandvik B and Zetterstrom R Test Meal in the Diagnosis of Malabsorption in Infancy Tolerance Tests Using Simultaneous Oral Administration of Glucose D Xylose Cream and Vitamin A 165
- Olegård R and Svennerholm L Effects of Diet on Fatty Acid Composition of Plasma and Red Cell Phospholipids in Three Month Old Infants 505
- Olin P and Ekholm R Carbimazole Treatment in Early Pregnancy Ultrastructural and Biochemical Observations on the Thyroid Glands of Two Twin Fetuses 565
- Olin P Studies on Thyroid Proteins in Childhood Goitre 578
- Pallisaard G and Goldschmidt E The Oculo Cerebro Renal Syndrome of Lowe in Four Generations of One Family 146
- Pennock C A Wharton B A and White F Urinary Glycosaminoglycan Excretion in the Neonatal Period 299
- Persson B and Tunell R Influence of Environmental Temperature and Acidosis on Lipid Mobilization in the Human Infant during the First Two Hours after Birth 385
- Rapola J and Savilahti E Immunofluorescent and Morphological Studies in Congenital Nephrotic Syndrome 253
- Reid M McC Reilly B J Murdock A I and Sway P R Cardiomegaly in Association with Neonatal Hypoglycaemia 295
- Roberts D F Rozner L M and Swan A V Age Menarche Physique and Environment in Industrial North East England 158
- Samánek M Houstek J Vavrova V Ruth C and Snobl O Distribution of Pulmonary Blood Flow in Children with Cystic Fibrosis 149
- Samuelson G An Epidemiological Study of Child Health and Nutrition in a Northern Swedish County III Medical and Anthropometrical Examinations 6
- Say B Balci S Pirnar T and Tuncbilek E A New Syndrome of Dysmorphogenesis Imperforata Associated with Poly Oligodactyly and Skeletal (Main Vertebral) Anomalies 197
- Schettini F Bratta A Mantone A and Zizzadoro F Acid Lysis of Red Blood Cells in Normal Children I Schuler D Schongut L Cserháti E Siegler J and Gács G Lymphoblastic Transformation Chromosome Pattern and Delayed Type Skin Reaction in Ataxia Telangiectasia 66
- Schwarze R Kintzel H W and Hinkel G K The Influence of Orotic Acid on the Serum Bilirubin Level of Mature Newborn 703
- Schweigsuth O Gerard Marchant R Plainfosse B Lemerle J Watchi J M and Serigne P Bilateral Non Functioning Thecoma of the Ovary in Epileptic Children under Anticonvulsant Therapy 6
- Sjakaalen P and Halvorsen S Inhibition of Erythropoiesis by Plasma from Newborn Infants 301
- Skrede S Stromme J H Stokke O Lie S and Ekjarm L Fetal Congenital Lactic Acidosis in Two Siblings II Biochemical Studies in Vivo and in Vitro 133
- Spennati G F Orzalesi M Bottini E and Pigram P Stability of Acid Phosphatase of Fetal Red Blood Cells during Incubation with Acetylphenylhydrazine 19
- Sterky G Kjellman O Hogberg O and Löfroth A L Dietary Composition and Dental Disease in Adolescent Diabetics A Pilot Study 461
- Szczepski O Walczak M Maciejewski J Bittner K Czekalski S and Waligora A The Behaviour of Some Indices of Calcium Phosphate Metabolism in Turner's Syndrome 73
- Sovik O Stromme J H and Folkers K Coenzyme Q in Duchenne Muscular Dystrophy A Preliminary Therapeutic Trial 428
- Tan K L The Third Fontanelle 329
- Tomovic E J Page Faulk W and Fudenberg H H Anaphylaxis and Red Cell Survival Studies in a Child with Insulin Resistant Diabetes Mellitus 647
- Tormá T and Donner M Hemispherectomy in Early Hemiplegia and Intractable Epilepsy 545
- De Vaan G A M Bakkeren J A J M Schretlen E D A M and Reerink H L Asparaginase Treatment of Acute Leukaemia in Children 22

Vahlquist B, Engner G and Sjogren I. Malnutrition and Size of the Cerebral Ventricle. Echoencephalographic Studies in Infants and Young Children. Preliminary Communication 533

apaavuo E. K. and Krohn K. Intensive Care of Small Premature Infants II Postmortem Findings 49

Isakorpi J. K. Palo J. and Renkonen O. V. The Incidence of PKU in Finland 606

Ogeli B, Riedwyl H, Donath A and Oetliker O. Comparison of Glomerular Filtration Rate and Effective Renal Plasma Flow Determinations Obtained by a Single Injection Technique and by Means of a Standard Clearance Technique in Children 579

Lamiet P. and Chunga F. Separation by Gel Filtration and Microdetermination of Unbound Bilirubin II Study of Sera in Icteric Newborn Infants 33

Zuppinger K. A. and Josa E. E. Influence of Long Term Growth Hormone Therapy on Glucose Tolerance and Insulin Secretion 678

Ohman R., Ekelund H. and Svennerholm L. The Diagnosis of Tay Sachs Disease 393

Ozoylu S. and Turhan, O. Alkaline Phosphatase Activity of Duodenal Juice in Rickets Due to Vitamin D Deficiency 338

Case Reports

Alexiou, D. Chrysostomidou O. Vlachos I. and Deligeorgis D. Trisomy 18 with Ovarian Dysgenesis 93

Batstone G. F. Cole A. P. and Sandry S. A. A Case of Tuberosc Sclerosis in the Newborn 349

Haahr J. and Halveg A. B. Congenital Leukaemia 720

Henniksson P., Nilsson I. M., Bergentz S. E., Ljungqvist U. and Rosengren B. Giant Haemangioma with a Disorder of Coagulation -

van der Horst J. L. and Wadman S. K. A Variant Form of Brain Red-Chain Keto Aciduria 594

Iancu T. and Chian E. Ectopic Spleen in an Rh Incompatible Infant 353

Aouba K., Jura J. and Zitová D. Hepatic Involvement in the Course of Acquired Toxoplasmosis 482

Palo J. and Iivanainen, M. The Cutis Verticis Gyrate and Mental Retardation Syndrome in a 4-Year Old Boy 346

Reimer S. H., Seelenfreund M. and Ben Bassat M. Cutis Lava Associated with Severe Intrauterine Growth Retardation and Congenital Dislocation of the Hip 357

Ridder M. A. C., Carrod O. and Berg J. M. A Case of Prader Willi Syndrome in a Child with a Small Extra Chromosome 22

Severi F., Tiepolo L. and Scappaticci, S. Identification of the Y Chromosome by the Fluorescence Technique in an XY/XO Gonadal Dysgenesis 716

Tayari K., Say B., Furt T. and Gursu, G. Oculodentodigital Dysplasia Syndrome 235

Tondeur M., Vámos-Hurwitz, E., Cremer N. and Loeb H. Mucopolysaccharidosis in a Three Months Old Infant. Clinical and Ultrastructural Studies 98

Wennevold A. and Kruangelbach, J. Prolonged Q T Interval and Cardiac Syncope 339

Review Articles

Dahlqvist A. and Landquist B. Lactose Intolerance and Protein Malnutrition 488

Eklöf O., Ekström G., Eriksson B. O., Michaelson M., Stephensen O., Söderlund S., Thoren C. and Wallgren G. Arterial Anomalies Causing Compression of the Trachea and/or the Oesophagus. A Report of 30 Symptomatic Cases 81

Harlund Christensen E. and Øster J. Adhesions of Labia Minora (Synchia Vulvae) in Childhood. A Review and Report of Fourteen Cases 709

Short Communications

Boda D., Murányi, L., Akorjaj I. and Veress I. Peritoneal Dialysis in the Treatment of Hyaline Membrane Disease of Newborn Premature Infants. Results of a Controlled Trial. Preliminary Report 90

Chrysostomidou C. M., Caslaris E., Alexiou D. and Bartsocas C. S. Trisomy 18 in Greece. Seven Cases of Pure Trisomy 18 and One with a D/G Translocation 591

Proceedings of Paediatric Societies

Proceedings of the Danish Paediatric Society Meetings Jan 14 Febr 11 March 11 April 8 May 13 1970 243

Proceedings of the Danish Paediatric Society Meetings Sept 9 Oct 14 Nov 11 Dec 7 Dec 11 1970 726

Proceedings of the European Society for Paediatric Endocrinology Meeting July 16-21 1970 603

Proceedings of the European Society for Paediatric Gastroenterology Meeting Aug 22-24 1970 363

Proceedings of the Finnish Paediatric Society Meetings Febr 14 April 24 1970 112

Proceedings of the Finnish Paediatric Society Meeting Febr 13 1971 602

Proceedings of the Finnish Paediatric Society Meeting April 3 1971 724

Proceedings of the Scandinavian Association of Paediatric Surgeons Meeting June 11-13 1970 495

Proceedings of the Swedish Paediatric Society Meetings April 26 Oct 10 1969 102

Supplements

Zweymüller Ernst and Preining, Othmar. The Insensible Water Loss of the Newborn Infant (Supplement 205)

Proceedings of the Sixteenth Scandinavian Paediatric Congress Turku August 2-August 5 1970 Edited by Matti Dahl (Supplement 206)

Olsson, Torsten and Victorin, Lars. Transthoracic Impedance with Special Reference to Newborn Infants and the Ratio Air-to-Fluid in the Lungs (Supplement 207)

Eeg-Olofsson, Orvar. The Development of the Electroencephalogram in Normal Children and Adolescents from the Age of 1 through 21 Years (Supplement 208)

Rune Valdemar. Acute Head Injuries in Children. An Epidemiologic Childpsychiatric and Electroencephalographic Study on Primary School Children in Umeå (Supplement 209)

- Rosberg Georg Parental Attitudes in Pediatric Hospital Admissions (Supplement 210)
- Eriksson Margareta Salicylate Induced Foetal Damage Late in Pregnancy An Experimental Study in Mice (Supplement 211)
- Hakulinen Alpo Urinary Excretion of Vanilmandelic Acid of Children in Normal and Certain Pathological Conditions (Supplement 212)
- Landtman Bernhard Clinical and Morphological Studies in Congenital Heart Disease (Supplement 213)
- Samuelson Gosta An Epidemiological Study of Child Health and Nutrition in a Northern Swedish County I Food Consumption Survey (Supplement 214)

- Berggreen Sheila Margaret A Study of the Mental Health of the Near Relatives of Twenty Multihandicapped Children (Supplement 215)
- Michelsons Katarina Cry Analyses of Symptomless Low Birth Weight Neonates and of Asphyxiated Newborn Infants (Supplement 216)
- Pediatric Work Physiology Proceedings of the Karolinska Institutet Symposia held in August 30-September 1 1970 Edited by Claes Thorén (Supplement 217)
- Rantakallio Paula The Effect of a Northern Climate on Seasonality of Births and the Outcome of Pregnancies (Supplement 218)

THE DECREASE IN THE SERUM BILIRUBIN LEVEL IN PREMATURE INFANTS BY OROTIC ACID

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The prevention of neonatal hyperbilirubinaemia has been the subject of intensive recent work in solving a search for inductors of the hepatic glucuronyl transferase system which might reasonably be used in a clinical setting. The use of phenobarbital (2, 18, 19, 20) in premature infants with disorders of respiration is not indicated because of the depressive effect of the barbiturate on respiration. The antenatal administration of phenobarbital to the mother, the effect of which on the newborn is also proved (11), meets with considerable difficulties in delivery before term. Edema develops with the use of phenylbutazone (4, 12) and it likely causes a displacement of the bilirubin out of its protein binding in the serum, therefore its clinical administration cannot be advised. It is true that nikethamide in a therapeutic dose is free from side effects, however its effect is too small to achieve an essential clinical advantage (6, 9, 16). Thus we have looked into other agents which might prevent an increase of the serum bilirubin into toxic ranges. Besides the immaturity of the glucuronyl transferase system, the deficiency of the uridine-coenzyme is also important in the genesis of the hyperbilirubinemia. The synthesis of the UTP passes from asparagine acid through the phase of orotic acid. Thus Brodersen recommended examination of the effect of orotic acid in newborn infants (1). This substance might induce the formation of glucuronyl

transferase because orotic acid is the precursor of the pyrimidine bases cytosine, thymine and uracil, which are necessary for the synthesis of the nucleic acids, and it was pointed out that the rate of synthesis of the RNA increases after the application of orotic acid (14). In addition to this substitution effect, there may also be an inductive effect of the enzyme. Up to now only Japanese authors (11) have investigated the influence of orotic acid on the bilirubin level of mature newborns. They found a small and partially significant decrease in the bilirubin level after treatment with a daily dose of 200 mg of orotic acid in a very small number of newborns.

In our first investigations we have therefore examined the influence of a medication with orotic acid. We administered a daily dose of 100 mg in premature infants from the 1st-6th day of life. The reaction of the coagulation factors was not influenced by this dosage; therefore we got no indication of an enzyme inductive effect. An influence on the serum bilirubin level was however detectable. 15 exchange transfusions were required in the control group (54 premature infants), whereas this was necessary in only 6 cases in the other group (56 premature infants) that has been treated with orotic acid (5). An increase in the dose of orotic acid seemed justifiable because we did not detect any side effects and because of the experiences that were gained in the animal ex-

Table 1 Distribution of birth weights

Birth weight	1 200- 1 500 g	1 500- 2 000 g	2 000- 2 500 g	Total
Orotic acid group	8	54	40	102
Control group	12	54	36	102

periment (3, 10, 17, 21) respectively, in the administration of orotic acid to adults (13). Therefore we increased the daily dosage of orotic acid up to 300 mg in our further investigations that are here discussed.

METHOD

Premature infants with a birth weight of 1 200-2 500 g were treated in the same manner as in the preceding investigations. One group of children was treated with a daily dose of 300 mg of orotic acid¹ from the first till the sixth day orally given in two single doses. The other group of children was not treated. Half of the children were chosen in an alternating manner and afterwards by the formation of groups whereby no selection was made. Feeding method and intensive care of the premature infants were practised identically in both groups. The total bilirubin as well as the indirectly reacting bilirubin in the serum was analysed according to the method of Jendrassik & Grof and Cleghorn respectively in all premature infants from the third till the sixth day of life. A blood exchange transfusion was performed at an increase in the indirectly reacting bilirubin of over 18 mg. In 10 premature infants of the control group and in 15 premature infants who had been treated with orotic acid a determination of the activity of the liver depending coagulation factors II and VII and of the factor V was performed (utilizing Behringwerke reagents) in each case on the fifth day.

RESULTS

A sum total of 223 premature infants was covered by the investigations. Out of 111 premature infants of the orotic acid group 9 children died from the first to the third day of life, so that 102 premature infants were at our disposal for the evaluation of the serum bilirubin levels and the calculation of the blood exchange frequency. Out of 112 premature infants of the control group 10 children died

within the first 3 days of life so that 102 premature infants without a treatment with orotic acid could be compared with the other group. The distribution of the birth weights in both groups showed no significant differences (Table 1).

Frequency of the blood exchange transfusion

An exchange transfusion had to be performed in only 4 children out of 102 premature infants of the orotic acid group, whilst this was necessary in 30 cases out of 102 premature infants of the control group. The difference in the exchange frequency of both groups is statistically most significant on an assumption of a probability of error of $p=0.1\%$ ($\chi^2=23.8$).

Reaction of the serum bilirubin

Arithmetic average value and standard deviation of the total and of the indirectly reacting

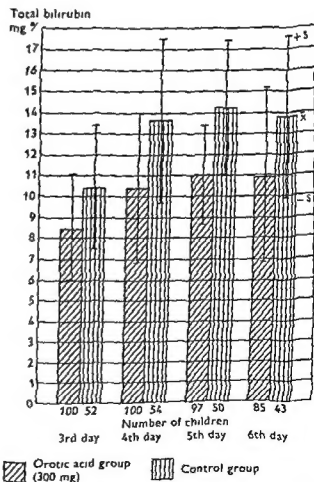


Fig. 1 Total bilirubin values in orotic acid treated group and in control group

A test preparation of the VEB Jenapharm

bilirubin were separately evaluated in the 3 weight groups for the 3rd-6th day of life. The bilirubin values performed after blood exchange transfusions were not taken into consideration in these calculations. Some high bilirubin levels of the control group must be omitted from the calculation for this group because blood exchange transfusions must be performed significantly more frequently in the premature infants of this group. There were no significant differences of the bilirubin levels between the 3 formed weight groups as well within the orotic acid group as within the control group. Therefore a total comparison of the average values of the orotic acid group and of the control group was possible. As it appears from the diagrams (Figs 1 and 2) the average values of the total and of the indirectly reacting bilirubin are distinctly lower in the orotic acid group from the 3rd-6th day of life. This dif-

Table 2 Reaction of coagulation factors

	F II	F VII	F V
<i>Orotic acid group 300 mg daily</i>			
<i>n=15</i>			
<i>x</i>	38	37	87
<i>s</i>	8.9	7.9	17.4
<i>Control group without orotic acid</i>			
<i>n=10</i>			
<i>x</i>	41	34	78
<i>s</i>	13.2	16.6	28.3

ference is statistically significant for the 3rd-6th day on an assumption of a probability of error of $p=1\%$ (t test)

Reaction of the coagulation factors

The activity of the liver-depending coagulation factors II and VII which was determined on the 5th day of life as well as the activity of factor V were equal in both groups (Table 2)

DISCUSSION

The results of our investigations show that action of orotic acid on serum bilirubin values could be essentially intensified by increasing the daily administered doses of orotic acid from 100 to 300 mg. The total and indirect bilirubin values on the 3rd-6th day of life are statistically significant lower than in the control group. Hereby must be considered that some high bilirubin values in the control group fall outside and cannot be brought into calculation because of performed exchange transfusions.

In respect to the essential blood exchange transfusions a very considerable decrease in the frequency could be achieved in the children who had been treated with orotic acid. In this group only 4 exchange transfusions were necessary whereas 30 exchange transfusions were required in the control group. The advantages of such a reduction are evident.

Before however an application of orotic acid can be generally advised the question of the side effects must be answered. As mentioned above orotic acid is a compound that the body

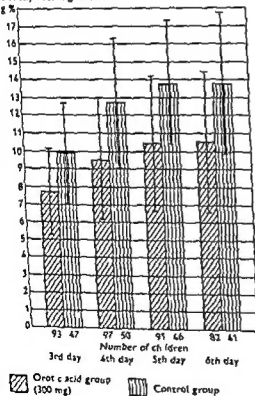
Indirectly reacting bilirubin
mg %

Fig. 2 Indirectly reacting bilirubin values in orotic acid treated group and in control group

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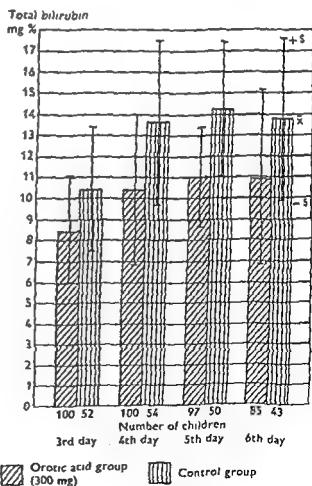


Fig. 1 Total bilirubin values in orotic acid treated group and in control group.

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orotic acid

itself synthesises in the synthesis of the nucleic acids. As the name suggests (oros = whey) orotic acid can also be found in cow's milk at an average of 50-100 mg per liter. A daily dose up to 3-5 g of orotic acid was administered to patients, who suffered from gout (13), without the observation of side effects. In the animal experiment, however, fatty degenerations of the liver (3, 10, 17, 21) and crystallisation of the orotic acid in the renal tubuli were detected after the administration of very high doses of orotic acid (15).

The clinical observation of the premature infants who had been treated with orotic acid in our hospital showed no detrimental effects. In the orotic acid group the mortality of 16 children from the 1st-10th day of life was practically on the same level with the mortality in the control group in which 15 children died. Pathologic anatomical control examinations were performed in the 7 premature infants of the orotic acid group who died within the 4th-10th day of life. No alterations were observed that could be charged to the use of orotic acid. We think, however, it is necessary to examine the question of side effects still more carefully before orotic acid is routinely administered to prevent premature infants from contracting hyperbilirubinemia. We ourselves have already started such studies which will also assist in deciding whether a renewed increase in the daily dose of orotic acid can be justified. It has been proved by our investigations that the effect of this substance depends upon the dose administered. The mode of action of the orotic acid is dubious up till now. Our investigations showed that the activity of the liver depending coagulation factors II and VII could not be increased by a dose of 300 mg compared with a control group. The coagulation factors and glucuronyl transferase are formed in the liver cell on the same spot, namely in the endoplasmatic reticulum. It has been proved that on application of the enzyme inductor phenobarbital there was an increase in the glucuronyl transferase activity simultaneously with an increase in the activity of the coagulation factors that

are formed in the liver (12). The absence of an increase in the activity of the coagulation factors that has been demonstrated by us, makes an inductive effect of the orotic acid not very likely. The results of the investigations of Klingner who did not find an enzyme inductive effect of the orotic acid in rats (8) correspond with our investigations. In our opinion the effect of the orotic acid is primarily a substitution effect on the coenzyme of the glucuronyl transferase, the UTP. With the co-operation of other clinics and institutes we are attempting to further elucidate this question.

SUMMARY

The influence of higher doses of orotic acid on the serum bilirubin level of premature infants was investigated following studies with a lower dose. 102 premature infants were treated with a daily dose of 300 mg of orotic acid from the 1st-6th day after birth. An equal number of children served as a control group. The serum level for the indirect bilirubin that was analysed from the 3rd-6th day of life could be statistically significantly decreased by the administration of orotic acid. Blood exchange transfusions were necessary only four times with the administration of orotic acid whereas blood exchange transfusions were required in 30 premature infants of the control group. The question of eventual side effects and the supposed mode of action of the orotic acid are discussed.

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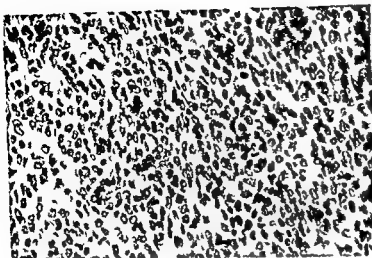


Fig 1 The tumor is made of plump fusiform cells with a tendency to a fasciculated or whorly arrangement Case 1 Hematoxylin phloxin saffron $\times 150$

cells with large rounded or elongated nuclei (Fig 1). There was no evidence of anaplasia or abnormal mitoses. In some areas the tumor was composed of dense cellular agglomerations lobulated by collagen septa. Elsewhere the cells were more widely separated by edema or hemorrhagic infarction and there were macrophages containing hemosiderin in the peripheral areas. None of the sections disclosed a rosette or folliculoid pattern suggestive of a granulosa cell component. Foot's stain revealed that individual tumor cells were surrounded by reticulin furnishing further confirmation of this impression (Fig 2). Intra and extra cellular lipid material was demonstrated by Sudan III on frozen sections (Fig 3). Normal remnants of ovarian tissue were not discernible. Sections of the uterus and oviducts were unavailable. Diagnosis: bilateral thecoma.

Case 2

Stella D. was referred to Children's Hospital at 31 years of age for generalized seizures. The attacks

were refractory to phenobarbital (40 mg daily) and as a result the child was placed on a combination of diphenylhydantoin and phenacetylurea. The convulsions were controlled however petit mal attacks supervened. Treatment was changed to phenobarbital (100 mg daily) and phenacetylurea (0.9 g daily). Toxic hypertrophy of the gums developed but treatment was not interrupted.

Four months later in February 1965 the child's mother noted a rapid increase of her daughter's weight. This was accompanied by facial edema and subsequent abdominal distension for which the child was readmitted to the hospital.

Physical examination revealed a well developed 6½ years old female (117 cm, 21 kg) with no evidence of precocious puberty. The abdomen was grossly distended and paracentesis produced 1500 ml of bloody fluid which contained lymphocytes and endothelial cells. The protein concentration was 3.6 g/100 ml. After this procedure a large suprapubic mass was palpable also found on rectal examination. The IVP and

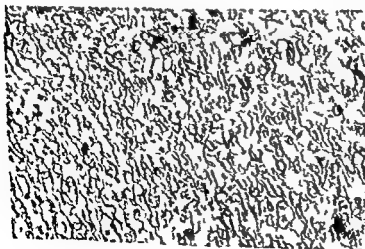


Fig 2 Individual cells are surrounded by reticulin fibers which do not demonstrate any follicular pattern Case 1 Foot's stain 600

BILATERAL NON FUNCTIONING THECOMA OF THE OVARY IN EPILEPTIC CHILDREN UNDER ANTICONVULSANT THERAPY

ODILE SCHWEISGUTH RÉMI GERARD MARCHANT BERNARD PLAINFOSSE
JEAN LEMERLE JEAN M WATCHI and PHILIPPE SERINGE

*From the Institut Gustave Roussy Villejuif and the Hopital des Enfants Malades
Paris France*

In a personal series of malignant ovarian tumors occurring in childhood we have observed two most unusual cases. These involved young girls who presented with bloody ascites associated with bilateral ovarian tumors. In each case surgical excision was curative and histological examination of the tumors revealed pure ovarian thecomas. Both children were epileptic and had been on long term anticonvulsant therapy.

The similarity of the clinical and pathological features of the 2 cases was so striking that it suggested a possible non fortuitous association between anticonvulsant therapy and the development of ovarian thecoma. This possibility was given further credence by our discovery of an almost identical case in the literature (1).

The purpose of this article is to acquaint pediatricians with our observations if indeed there is an association between epilepsy or which is more probably anticonvulsant therapy and the development of ovarian thecoma in childhood it may be anticipated that similar cases will be reported.

CASE REPORTS

Case 1

Maud H (I G R 12/60 2465) a 53 year old female had generalized seizures at 9 months of age and again at 19 months of age. Initial treatment with phenobarbital 40 mg daily was given. However at

3 years of age she developed petit mal seizures for which she was started on trimethadione 0.6 g daily. Phenacetylene, 0.9 g daily was subsequently added for better control of the petit mal. The child had been on this regimen for 6 months when she was referred to our hospital in April 1960 because of ascites. This had been detected 3 days prior to admission in connection with an episode of transient facial edema.

Physical examination revealed a normally developed 53 year old girl (108 cm 19.5 kg) with no evidence of abnormal sexual development. The abdomen was grossly distended the left iliac fossa being somewhat firmer to palpation than the right. The remainder of the physical examination was unremarkable. Two abdominal paracenteses prior to admission had effected the removal of a total of 1300 ml of bloody fluid. A third paracentesis of 1000 ml following admission permitted palpation of a large tumor in the left iliac fossa. The fluid contained numerous red cells macrophages and endothelial cells and a few clusters of basophilic cells suggesting malignancy. The protein concentration was 3.2 g/100 ml.

The IVP and chest film were normal as was examination of the bone marrow. Coagulation studies revealed normal coagulation and bleeding times as well as a normal prothrombin complex. Clot retraction was absent and clot lysis occurred in 30 min at 37°C. The test for fibrinolysis revealed it was almost complete in 2 hours.

Laparotomy disclosed bilateral solid ovarian tumors with distinct areas of hemorrhage and infarction. The tumors measured 10×12 cm and 6×9 cm on the left and right sides respectively. Bilateral oophorectomy and salpingectomy with subtotal hysterectomy were performed. Uterine size was normal and there was no evidence of abdominal metastasis. The postoperative course was uneventful and the coagulation abnormalities were corrected immediately. The child is well 10 years later with good control of the epilepsy.

Microscopic findings of both tumors were similar they were composed of bundles of plump spindle

Table 1 *Anti epileptic drugs given to the three patients*

Drug	Trade name	Faber's case		
		Case 1	Case 2	case
Phenobarbital	Phenobarbital	+	+	+
Trimethadione	Tridione	+	0	+
Paramethadione	Paradione	0	0	+
Phenacetylurea	Phenacemide	+	+	II
Phensuximide	Milontin	0	0	+
Diphenylhydantoin	Dilantin	0	+	+

she was treated with diphenylhydantoin and phenobarbital before being changed to para-dione (2). She was admitted to the hospital in August, 1949 with orbital and pedal edema abdominal ascites developed subsequently. The presence of a malar butterfly rash in addition to hematuria and proteinuria initially suggested drug toxicity. Following paracentesis a large supra pubic mass became palpable. At laparotomy bilateral tumors were resected with preservation of the uterus. Pathological examination of the ovaries was similar to that of our own cases and also consistent with the diagnosis of thecoma. The child has been followed for 10 years and has remained well with the exception of refractory petit mal seizures.

Ovarian thecoma is a neoplasm which develops from the ovarian mesenchyme. It is most often associated with morphological evidence of granulosa cell development. In cases of 'pure' thecoma two different pathological entities must be ruled out: thecal hyperplasia and fibroma of the ovary.

The diagnosis of thecal hyperplasia was proposed by the Armed Forces Institute of Pathology (Washington D.C.) on the basis of their review of the slides of our second case. They pointed out that "these ovarian changes would occur in association with torsion of the ovarian adnexal structure the ovary becoming tremendously enlarged secondary to venous obstruction" (8). Adnexal torsion was not observed in any of the three cases and therefore we are reluctant to accept this diagnosis.

We also entertained the diagnosis of fibroma of the ovary however several inconsistencies

mitigated against this diagnosis as well as areas of hemorrhage were more prominent in our cases than would normally be expected in ovarian fibroma. A fasciculated pattern of the cells was not evident the tumor cells were plump and cytologically quite distinct from fibroblasts. Finally in case 1 as in Faber's case special stains for intra and extra cellular lipids were positive the presence of fat in the mesenchymal ovarian tumor is generally accepted as the best microscopic criterion in support of the diagnosis of thecoma as opposed to fibroma.

Although bilateral theca granulosa cell tumors associated with precocious puberty are well known (10) Faber's case is the only case of bilateral pure thecoma without endocrine activity we have found in the pediatric literature.

The lack of feminizing activity in these 3 cases of pure ovarian thecoma is difficult to explain. The nature of hormonal synthesis in ovarian tumors is not well understood. Ryan (6) has postulated that thecal cells require the adjuvant presence of granulosa lutein cells or other sources of progesterone for them to become functionally active. Thorough examination of the pathological material of our cases failed to reveal a granulosa-cell component, and this could explain the lack of endocrine symptomatology.

The children under discussion were epileptic two suffered from grand mal and all three of them from petit mal epilepsy. None of the children revealed neurological evidence of an organic brain disorder. We are unaware of a genetic relationship between ovarian tumors and epilepsy. Miller & Fraumeni (5) in reviewing the charts of children with ovarian tumors found only one case in which a teratoma of the ovary was associated with microcephaly mental retardation and epilepsy. In our personal experience with 40 cases of ovarian tumors in children only the 2 cases of pure thecoma were associated with epilepsy. Nevertheless we recognize that a relationship between some cerebral anomalies and ovarian tumors cannot be entirely ruled out, because

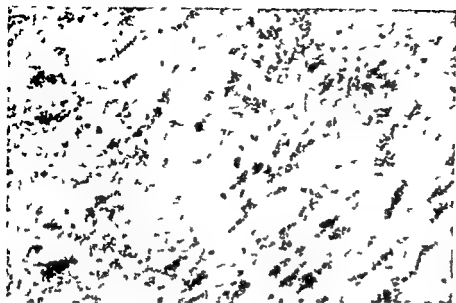


Fig 3 Lipidization is obvious most of the fat droplets are intra cellular Case 1 Sudan III on frozen section $\times 150$

chest X ray were within normal limits as were routine examinations of the blood and urine

At laparotomy it was apparent that the residual ascites was associated with bilateral ruptured ovarian tumors. Bilateral oophorectomy and subtotal hysterectomy were performed the remainder of the abdominal cavity was unremarkable. The post operative course was uncomplicated except for incomplete control of the epilepsy and the child is doing well 2 years later. A test for lymphoblastic transformation of lymphocytes *in vitro* in the presence of diphenylhydantoin was negative.

The pathological specimen consisted of bilateral ovarian tumors weighing 90 and 100 g respectively both revealed gross areas of hemorrhagic infarction (Fig 4). The microscopic appearance was similar to that of the first case and also there was no evidence of a granulosa cell component. Specially stained sections did not reveal the presence of lipid material in

the available sections. Remnants of normal ovarian tissue with normal primary follicles were discernible in one area. The uterus was of normal size with an immature endometrium and no evidence of glandular or stromal hyperplasia. The oviducts were normal. Diagnosis: bilateral thecoma.

DISCUSSION

A case almost identical to the above two has been reported by Faber (1).

A 3-year old girl developed petit mal epilepsy in December, 1948. She was treated with tri-methadione from January to March, 1949 following which she received a short course of succinimide. Between March and August 1949

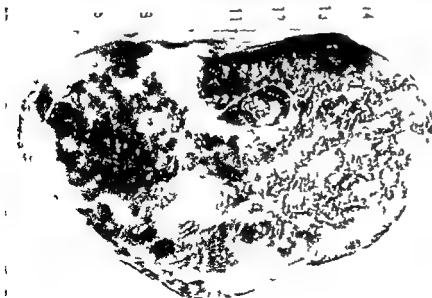


Fig 4 Gross section of the left tumor in case 2. Numerous pseudocystic areas after infarction and hemorrhages.

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Phensuximide	Milontin	0	0	+
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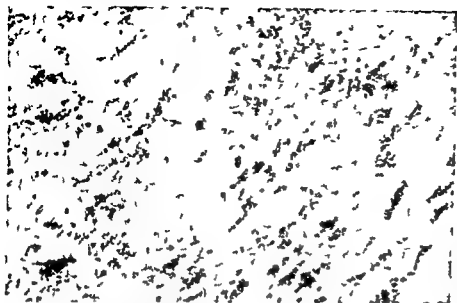


Fig 3 Lipidization = obvious most of the fat droplets are intracellular Case 1 Sudan III on frozen section $\times 150$

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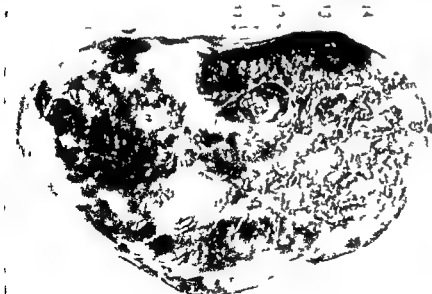


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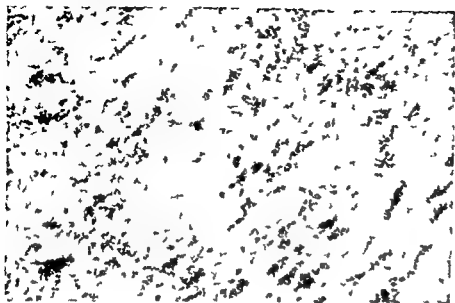


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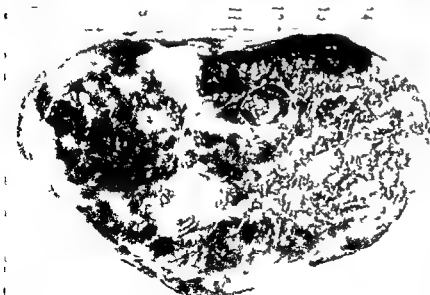


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MILD AND SEVERE PKU COMPARATIVE STUDIES IN TWO INFANTS

O HJALMARSSON, R. JAGENBURG and S. RÖDIER

From the Departments of Paediatrics and Clinical Chemistry
University of Gothenburg Gothenburg Sweden

With the introduction of massscreening surveys of newborns for early detection of phenylketonuria an increasing number of children with hyperphenylalaninemia is detected. Not only the incidence of classical phenylketonuria (PKU) has been found to be greater than previously regarded (6) but a considerable number of cases have been found to have a moderate increase of the blood phenylalanine level which has not always led to the serious consequences seen in children with untreated PKU (1, 2, 3, 7, 10, 12, 18, 19, 20). Many of these cases no doubt differ from classical PKU but there is no agreement about the classification of the different forms of hyperphenylalaninemia. WHO recommended in 1968 (24) a classification into four groups:

1. classical PKU—here called *classical severe PKU*
2. classical PKU with 2-3 times greater tolerance of phenylalanine than in the previous form—here called *classical mild PKU*
3. *transient hyperphenylalaninemia* with or without phenylpyruvicaciduria and with or without mental retardation and
4. *persistent hyperphenylalaninemia* without phenylpyruvicaciduria or mental retardation

This classification is based on both clinical and/or laboratory findings. There are no definite limits for the plasma phenylalanine level separating the groups because the level varies

considerably with age and the phenylalanine intake.

This paper describes a study of two infants with hyperphenylalaninemia detected during their first week of life. One of them was a girl with a classical severe PKU. She had a very high blood concentration at 5 days of age and diet therapy was immediately started. The other infant had slowly increasing plasma phenylalanine values amounting to 16 mg/100 ml at 3 weeks of age. There was no phenylpyruvic aciduria. The changes in the plasma phenylalanine level during fasting after ordinary meals and after intravenous phenylalanine infusions were determined in order to disclose any differences in the phenylalanine turn over in the two infants. The studies were performed when the infants were between 2-5 weeks of age.

CASE REPORTS

Case 1. This girl is the third child of healthy non-consanguineous parents. No mental retardation or disease is known in the family. The patient's two siblings are normal. The infant was born at term, birth weight 3300 g. The gestation and the delivery were normal. The infant was initially breast fed. A Guthrie test for PKU at 5 days of age showed a blood phenylalanine level of more than 20 mg/100 ml increasing to 47 mg/100 ml before treatment was started at the age of 11 days. Other amino acids including tyrosine were normal. The phenylalanine content of the food had to be considerably reduced to reach a suitable blood level (Fig. 1). From the third month of life a phenylalanine intake of about

complete neurological and radiological studies were not performed in our reported cases

The role of anti-convulsant therapy in the development of ovarian thecoma is more interesting to consider. Table 1 lists the drugs received by our patients. These drugs have been reported as able to produce pseudo lymphomas (7), true lymphomas (4), and even pseudo pseudo lymphomas (3). Hydantoin derivatives are the most frequently implicated. These similar side effects illustrated by the above group may possibly be explained on basis of the common structural features (phenol group) (9) present in most of the anti convulsant drugs.

Both in the cases of the lymphomatous and ovarian growths found in patients treated with anti convulsant drugs the question can be discussed whether there is a true or a pseudo tumor.

It is certain, however that if ovarian thecomas indeed represent a side effect of anti convulsant therapy they are extremely rare in proportion to the large number of patients on treatment for epilepsy.

According to the low malignant potential of granulosa theca cell tumors the outlook for survival in these cases has been good with surgery alone. Hysterectomy was unnecessary in our 2 cases and it should be avoided but the surgeons were impressed by the gross appearance of the ovaries and their association with bloody ascites.

Bilateral oophorectomy is quite undesirable in a young female and one wonders whether withdrawal of anti convulsant therapy might permit the regression of the ovarian changes as has been described in connection with pseudo lymphomatous adenopathy. If one could be relatively certain of the clinical diagnosis this would be a valid trial however the rapid evolution of clinical signs and symptoms as well as the striking changes of the ovaries noted at laparotomy tend a priori to make the regression of the tumors rather dubious. In any case we believe that surgery should be as conservative as possible.

SUMMARY

The peculiar features of two bilateral thecoma of the ovary are described. They were discovered because of a rapid enlargement of the abdomen with hemorrhagic ascites, but without any sign of precocious puberty. They occurred in young females treated with anti-convulsant therapy for epilepsy. They have been cured with surgery alone.

A third case has been found in the literature. Discussion is raised about the meaning of this association. The possible relationship with side effect of anti epileptic drugs is suggested.

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MILD AND SEVERE PKU COMPARATIVE STUDIES IN TWO INFANTS

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With the introduction of massscreening surveys of newborns for early detection of phenylketonuria an increasing number of children with hyperphenylalaninemia is detected. Not only the incidence of "classical" phenylketonuria (PKU) has been found to be greater than previously regarded (6) but a considerable number of cases have been found to have a moderate increase of the blood phenylalanine level which has not always led to the serious consequences seen in children with untreated PKU (1, 2, 3, 7, 10, 12, 18, 19, 20). Many of these cases no doubt differ from classical PKU but there is no agreement about the classification of the different forms of hyperphenylalaninemia. WHO recommended in 1968 (24) a classification into four groups:

- 1 classical PKU—here called *classical severe PKU*
- 2 classical PKU with 2-3 times greater tolerance of phenylalanine than in the previous form—here called *classical mild PKU*
- 3 *transient hyperphenylalaninemia* with or without phenylpyruvic aciduria and with or without mental retardation and
- 4 *persistent hyperphenylalaninemia* without phenylpyruvic aciduria or mental retardation

This classification is based on both clinical and/or laboratory findings. There are no definite limits for the plasma phenylalanine level separating the groups because the level varies

considerably with age and the phenylalanine intake.

This paper describes a study of two infants with hyperphenylalaninemia detected during their first week of life. One of them was a girl with a classical severe PKU. She had a very high blood concentration at 5 days of age and diet therapy was immediately started. The other infant had slowly increasing plasma phenylalanine values amounting to 16 mg/100 ml at 3 weeks of age. There was no phenylpyruvic aciduria. The changes in the plasma phenylalanine level during fasting after ordinary meals and after intravenous phenylalanine infusions were determined in order to disclose any differences in the phenylalanine turn over in the two infants. The studies were performed when the infants were between 2-5 weeks of age.

CASE REPORTS

Case A J This girl is the third child of healthy not consanguineous parents. No mental retardation or disease is known in the family. The patient's two siblings are normal. The infant was born at term, birth weight 3300 g. The gestation and the delivery were normal. The infant was initially breast fed. A Guthrie test for PKU at 5 days of age showed a blood phenylalanine level of more than 20 mg/100 ml increasing to 47 mg/100 ml before treatment was started at the age of 11 days. Other amino acids including tyrosine were normal. The phenylalanine content of the food had to be considerably reduced to reach a suitable blood level (Fig. 1). From the third month of life a phenylalanine intake of about

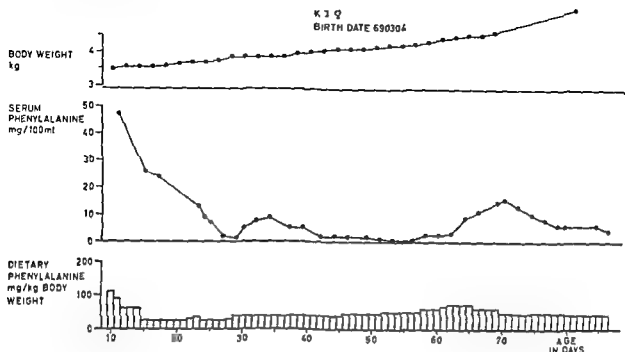


Fig 1 Weight curve blood phenylalanine level and calculated phenylalanine intake in patient K J

40 mg per kg and day gave a blood level of 4-7 mg/100 ml. The patient is now 10 months old. The weight gain, her mental and neurological status and EEG registrations are normal.

Case S S This girl is the third child of healthy parents. One possibly two of her maternal grandfather were mentally retarded, had pareses and died before 20 years of age. The parents are not consanguineous and the siblings are healthy and normally developed. The fasting plasma phenylalanine level is normal in the parents and the siblings. The infant was born at term, birth weight 3 300 g. The gestation and the delivery were normal. She was admitted to hospital 9 days old because a phenylalanine concentration of 10 mg/100 ml had been found by

the Guthrie method in a blood sample obtained at the age of 5 days. During the first 20 days of life the girl was breast fed ad lib. The calculated phenylalanine intake was between 120 and 160 mg per kg body weight and day (Fig 2). On this diet the blood phenylalanine level slowly increased from 10 to 16 mg/100 ml. Apart from the elevated phenylalanine level the plasma amino acids including tyrosine were normal. Phenylpyruvic acid, *o*-hydroxyphenylacetic acid and phenylacetylglutamine were not found in the urine. On account of the increasing level of phenylalanine in the blood the intake of this amino acid was reduced to 80 mg per kg and day by replacing half the milk volume by Lofenalac® which resulted in an immediate decrease of the phenylalanine level.

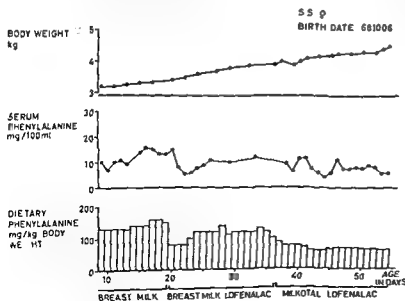


Fig 2 Weight curve blood phenylalanine level and calculated phenylalanine intake in patient S S

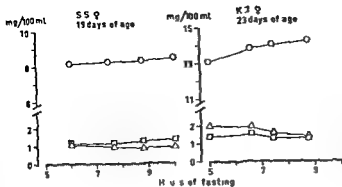


Fig 3 Changes in the plasma levels of phenylalanine (○—○) tyrosine (△—△) and leucine (□—□) during fasting. Zero time corresponds to 2 o'clock in the night when the fast meal was given

to 6 mg/100 ml. The phenylalanine intake was increased to about 120 mg per kg and day during the following 12 days and this gave a serum level of about 10 mg/100 ml. To reach a level of 4-7 mg/100 ml the intake was later reduced to about 60 mg phenylalanine per kg and day. The weight gain and somatic development during the period of study were normal. At the age of 4 weeks a slight muscular hypertonia without definite pathological significance was noticed. EEG registrations were normal. Eight weeks old the infant was discharged from the hospital and received continued dietary treatment at home. During the third month of life the phenylalanine intake had to be gradually reduced to about 40 mg per kg and day to keep the blood level within 4-7 mg per 100 ml.

METHODS

Phenylalanine was either measured by the Guthrie method (8) for daily routine (Figs 1 and 7) or by

ion exchange chromatography (Figs 3-6) using a Beckman amino acid analyzer model 120 C (21). A short program specially adapted for determination of tyrosine and phenylalanine was run. For these determinations blood was obtained by internal jugular vein puncture. Heparin was used as an anticoagulant. The plasma proteins were precipitated by picric acid (22). The urine was analyzed for the presence of phenylpyruvic acid by Phenistix[®] and by the α keto acid test (9). The urinary excretion of 3-hydroxyphenylacetic acid and phenylacetylglutamine was determined by paper chromatography (4).

RESULTS

Plasma phenylalanine level during fasting. In neither of the patients the plasma phenylalanine level decreased during fasting (Fig 3). Instead there was a slight increase amounting to 0.3 mg/100 ml during 4 hours (1% increase).

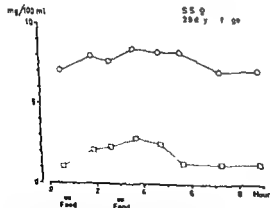


Fig 4 The influence of food on the plasma levels of phenylalanine (○—○) and leucine (□—□) in patient S.S. Zero time corresponds to 8:00 a.m. Meals were given at 2 o'clock in the night and at the times indicated in the figure. Each meal was calculated to contain 110 mg of phenylalanine.

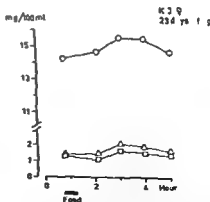


Fig 5 The influence of food on the plasma levels of phenylalanine (○—○) tyrosine (△—△) and leucine (□—□) in patient K.J. Zero time corresponds to 10:00 a.m. Meals were given at 2 o'clock in the night and at the time indicated in the figure. The meal was calculated to contain 50 mg of phenylalanine.

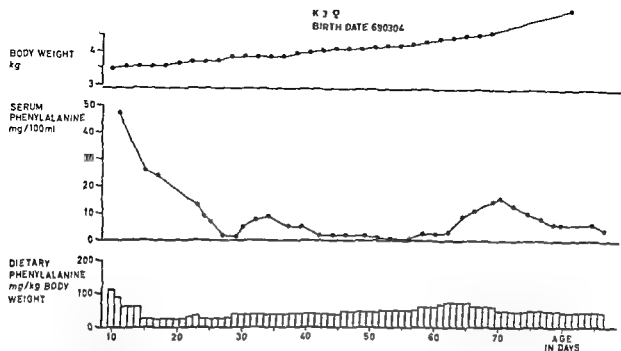


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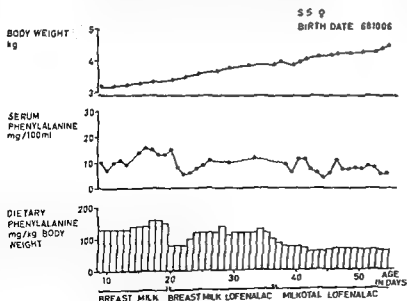


Fig 2 Weight curve blood phenylalanine level and calculated phenylalanine intake in patient S S

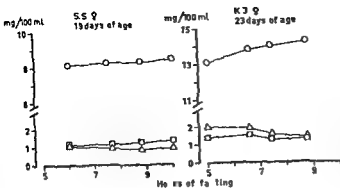


Fig 3 Changes in the plasma levels of phenylalanine (○—○) tyrosine (△—△) and leucine (□—□) during fasting. Zero time corresponds to 2 o'clock in the night when the last meal was given

to 6 mg/100 ml. The phenylalanine intake was increased to about 170 mg per kg and day during the following 12 days and this gave a serum level of about 10 mg/100 ml. To reach a level of 4–7 mg/100 ml the intake was later reduced to about 60 mg phenylalanine per kg and day. The weight gain and somatic development during the period of study were normal. At the age of 4 weeks a slight muscular hypertonia without definite pathological significance was noticed. EEG registrations were normal. Eight weeks old the infant was discharged from the hospital and received continued dietary treatment at home. During the third month of life the phenylalanine intake had to be gradually reduced to about 40 mg per kg and day to keep the blood level within 4–7 mg per 100 ml.

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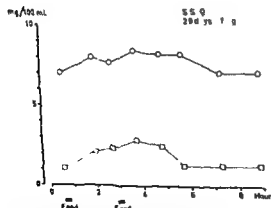


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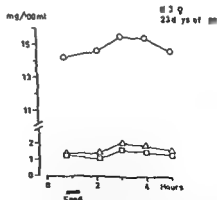


Fig 5 The influence of food on the plasma levels of phenylalanine (○—○) tyrosine (△—△) and leucine (□—□) in patient K.J. Zero time corresponds to 10.00 a.m. Meals were given at 0 o'clock in the night and at the time indicated in the figure. The meal was calculated to contain 50 mg of phenylalanine.

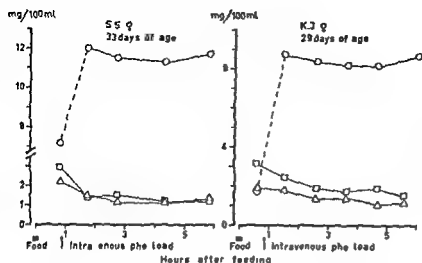


Fig 6 Changes in the plasma levels of phenylalanine (○—○), tyrosine (△—△) and leucine (□—□) after an intravenous single injection of phenylalanine. S S was given 40 mg and K J 53 mg per kg body weight. The loading was performed 45 min after the second meal of the day.

per hour) in patient S S and 1.1 mg/100 ml (2% per hour) during 4 hours in patient K J. During the fasting period the urinary loss of phenylalanine was less than 0.1 mg per hour in both patients. The phenylalanine loss by the urine in patient S S was less than 5 mg per day even when the plasma level was at about 15 mg/100 ml.

Changes in the plasma phenylalanine level after feeding. In patient S S the change in the plasma phenylalanine level after feeding was followed from 8.30 a.m. to 4.30 p.m. (Fig 4). Before the study the child was given the ordinary night meal at 2.00 a.m. During the study two ordinary meals of breast milk were given at 8.40 and 10.55 a.m. respectively. Each meal was calculated to contain 70 mg of phenylalanine. There was a slight increase in the phenylalanine level of about 1 mg per 100 ml plasma after each meal with a slow return to the fasting value. The fasting level obtained at 8.30 in the morning was reached 4 hours after the second meal. In patient K J the plasma phenylalanine level was followed after one of the meals (Fig 5) containing about 50 mg of phenylalanine. The changes observed were similar to those in patient S S.

Intravenous phenylalanine loading. The loading was performed 45 min after the second meal of the day by a rapid infusion of a 2.5% solution of L-phenylalanine (kindly supplied by AB Astra Södertälje) into a scalp vein. The increase in the plasma phenylalanine level from

7.2 to 12.0 mg/100 ml (150 mg phenylalanine injected) after 1 hour in patient S S and from 1.7 to 8.7 mg/100 ml (200 mg phenylalanine injected) in patient K J (Fig 6) corresponded to an apparent distribution volume of 3.1 l (body weight 3.82 kg) and 2.9 l (body weight 3.80 kg) respectively.

The plasma disappearance rate calculated from the decrease in the plasma phenylalanine level between 2 and 3 hours after the loading i.e. about 3 to 4 hours after the last meal, was in both patients less than 0.2 mg/100 ml per hour. As in the previous tests the plasma phenylalanine level thereafter increased. The tyrosine level did not increase after the loading in either of the infants.

DISCUSSION

The diagnosis of classical severe PKU is generally based on the following criteria (5, 11): a normal phenylalanine level at birth increasing to 30 mg/100 ml or higher at the end of the first week of life but without increase in the tyrosine level; the presence of *o*-hydroxyphenylacetic acid and later phenylpyruvic acid in the urine. A complete lack of phenylalanine hydroxylase activity has been noted in patients on whom liver biopsy has been performed (10, 13, 14, 15, 17, 23). In addition to this severe form of PKU other apparently genetic variants of hyperphenylalaninemia are described (3, 10, 12, 16, 18, 24). Classical mild PKU also

called atypical PKU is characterized by a slow rise in the plasma phenylalanine level and a late appearance of phenylpyruvic aciduria. Dietary treatment is considered necessary. Another variant called persistent hyperphenylalaninemia is generally not accompanied with phenylpyruvic aciduria because the plasma phenylalanine level is only moderately elevated. Mental retardation and other neurological symptoms are considered not to occur (24). In three patients considered to have persistent hyperphenylalaninemia a marked decrease but not an absence of phenylalanine hydroxylase activity have been reported (10-14). In 1 case with a mild form of classical PKU no activity of the enzyme was observed (14). The reason for the difference in the dietary phenylalanine tolerance in severe and mild PKU is unknown. Oral phenylalanine tolerance tests do not seem to be suitable for differentiating between the two forms of hyperphenylalaninemia (3-7).

Of the 2 cases studied by us patient K J no doubt suffered from severe classical PKU. Patient S S had a greater dietary tolerance for phenylalanine and could either be viewed as having a mild form of classical PKU or persistent hyperphenylalaninemia. The blood phenylalanine level never exceeded 16 mg per 100 ml in spite of the fact that the girl for the first 20 days of life was fed breast milk ad libitum. Phenylpyruvic acid was not detected in the urine and *o*-hydroxyphenylacetic acid only after phenylalanine loading. This difference in the dietary phenylalanine tolerance between the two infants is most easily explained assuming a persistent phenylalanine hydroxylase activity in patient S S. Patient K J—as a case of classical severe PKU—was considered to have a complete lack of enzyme activity (1). However the results obtained gave no definite evidence for a persistent phenylalanine hydroxylase activity in patient S S. The changes of the phenylalanine levels during fasting after ordinary meals and after intravenous phenylalanine loadings were similar in the two patients. The only difference observed was an insignificantly greater increase in the plasma

phenylalanine level during fasting in patient K J (Fig. 3). We have not had the opportunity to perform direct measurements of the enzyme activity on a liver biopsy. The fact that the plasma phenylalanine level increased during fasting shows that the excretion and metabolism of phenylalanine was less than the amount of phenylalanine liberated by breakdown of protein during the catabolic phase. The results obtained emphasize the difficulties in getting relevant information from the plasma disappearance rate of an amino acid even after intravenous infusions as infants when fasting soon turns into a catabolic state.

The clear increase in the plasma phenylalanine after milk feeding (Figs. 4 and 5) and the fact that the urinary excretion of phenylalanine was small make a defect intestinal absorption or a reduced renal tubular reabsorption improbable as the cause of the greater dietary phenylalanine tolerance in patient S S.

Mutation of a structure gene causes a change in the structure of a specific protein. If this protein is an enzyme the result can be either a partial or a complete loss of enzyme activity. Such a genetic modification of an enzyme molecule can change its K_m value. A high K_m value of the phenylalanine hydroxylase in patient S S might explain why the plasma phenylalanine level did not rise to the excessive high values seen in severe classical PKU. We have not been able to test this hypothesis as the family has moved to another part of Sweden. The fact that the phenylalanine intake had to be gradually reduced to about 40 mg per kg and day when the child was 3 months old to reach a suitable plasma phenylalanine level is not inconsistent with this hypothesis.

As mentioned above there are no doubt several genetic variants of hyperphenylalaninemia. Kinetic studies of the enzyme with determinations of K_m and V_m , and probably also genetic studies have to be made to characterize the different forms of hyperphenylalaninemia. Such studies have not yet been performed. Therefore we have to rely on more empirical

classifications. According to the present classification proposed by WHO (24) the atypical case 55 ought to be viewed as having a mild form of classical PKU. Diet therapy was considered necessary with regard to the present minor knowledge about the causes of mental retardation in PKU.

SUMMARY

Two infants with hyperphenylalaninemia first recognized at the age of 5 days were studied. One of the patients was a case of classical severe PKU. The other patient, on an unrestricted diet, showed only a slow rise in the plasma phenylalanine level amounting to 16 mg/100 ml at 3 weeks of age and had no phenylpyruvic aciduria. The plasma phenylalanine levels of the two infants were determined during fasting, after feeding and after intravenous phenylalanine loading. The results obtained did not reveal any significant difference in the phenylalanine turn over between the two infants.

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ACID LYSIS OF RED BLOOD CELLS IN NORMAL CHILDREN

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The human foetal red cell is different from that of the adult because of its surface morphology (16) ultrastructure (33) size (17) mechanical (5) and osmotic fragility (7 8 31 34 43 45) heat (3 9) and acid resistance (22). The membrane permeability is also different and in foetal red cells the thiourea and the glycerol have a slow penetration (14 15) the water has a slow diffusion (1) and the glucose shows a greater permeability (47). However the rate of lysis in hypotonic solutions of glucose is similar in foetal and in adult red cells (40).

The foetal red cell contains predominantly haemoglobin F a lower amount of acetylcholinesterase (14 21 26) and a higher glucose 6 phosphate dehydrogenase activity (26 38) and it shows a different electrophoretic mobility (14 37).

The activities of carbonic anhydrase (44) of catalase (20 26) of phosphofructokinase (6 25 30) and of NADH methemoglobin reductase (24 25 35 36) are reduced so as a relative deficiency of membrane adenosine triphosphatase (46) is present. Pyruvate kinase (26) lactic dehydrogenase (26) fructose 1 6-diphosphate aldolase (26) enolase (30) phosphoglycerokinase (30) activities are increased. The glycolytic intermediates phosphoenolpyruvate and 2-3 diphosphoglycerate are reduced (30). The levels of ATP and glucose consumption are higher (29) and ATP and glutathione in stabilites are greater (29) in newborn red cells.

Professor of Child Health

The erythrocytes of newborn infants lose potassium at an accelerated rate when stored at 4 C or when incubated at 37 C (4 43) for a decreased active potassium influx (4).

Foetal cells show a higher reactivity when tested with antigloboside and anti hematoside serum (2).

The characteristics of the foetal red cells are not yet fully evaluated especially in order to the period of life when the functional maturation is complete.

Acid lysis of red cells is probably a very indicative index of chemical changes in the membrane accompanying ageing red cells (10) and of its water content (13 28).

In view of this problem acid lysis of the foetal and adult erythrocytes was studied by means of an automated procedure.

MATERIALS AND METHODS

Capillary blood obtained by finger prick from full term newborns aged from 1 to 6 days from infants aged from 7 to 360 days and from children aged from 2 to 10 years was used. All the subjects were healthy.

The samples of blood were diluted 1:30 (20 µl of blood and 60 ml of isotonic NaCl solution buffered to pH 7.3 by veronal HCl).

Acid haemolysis was obtained by submitting the red blood cell suspension to a progressive increasing hydrogen ion concentration by dialysis against an isotonic solution of low pH (10).

The degree of haemolysis as a function of continuously reduced pH was automatically recorded by means of the Fragiligraph (model D 2 Elron Electronic Ind Haifa, Israel).

A sample of 40 µl of the red cell suspension was introduced into a microcuvette of 0.04 ml capacity.

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Key words: Inborn errors of metabolism, phenylalanine, phenylketonuria.

ACID LYSIS OF RED BLOOD CELLS IN NORMAL CHILDREN

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The human foetal red cell is different from that of the adult because of its surface morphology (16) ultrastructure (33) size (17) mechanical (5) and osmotic fragility (7) 31 34 43 45 heat (3 9) and acid resistance (22). The membrane permeability is also different and in foetal red cells the thiourea and the glycerol have a slow penetration (14 15) the water has a slow diffusion (1) and the glucose shows a greater permeability (47). However the rate of lysis in hypotonic solutions of glucose is similar in foetal and in adult red cells (40).

The foetal red cell contains predominantly haemoglobin F, a lower amount of acetylcholinesterase (14 21 26) and a higher glucose 6-phosphate dehydrogenase activity (26 38) and it shows a different electrophoretic mobility (14 37).

The activities of carbonic anhydrase (44) of catalase (20 26) of phosphofructokinase (6 23 30) and of NADH-methemoglobin reductase (24 25 35 36) are reduced so as a relative deficiency of membrane adenosine triphosphatase (46) is present. Pyruvate kinase (26) lactate dehydrogenase (26) fructose 1 6-diphosphate aldolase (26) enolase (30) phosphoglycerokinase (30) activities are increased. The glycolytic intermediates phosphoenolpyruvate and 2-3 diphosphoglycerate are reduced (30). The levels of ATP and glucose consumption are higher (29) and ATP and glutathione in stabilities are greater (29) in newborn red cells.

The erythrocytes of newborn infants lose potassium at an accelerated rate when stored at 4 °C or when incubated at 37 °C (4 43) for a decreased active potassium influx (4).

Foetal cells show a higher reactivity when tested with antigloboside and anti hematoside serum (2).

The characteristics of the foetal red cells are not yet fully evaluated especially in order to the period of life when the functional maturation is complete.

Acid lysis of red cells is probably a very indicative index of chemical changes in the membrane accompanying ageing red cells (10) and of its water content (13 28).

In view of this problem acid lysis of the foetal and adult erythrocytes was studied by means of an automated procedure.

MATERIALS AND METHODS

Capillary blood obtained by finger prick from full term newborns aged from 1 to 6 days from infants aged from 7 to 360 days and from children aged from 2 to 10 years was used. All the subjects were healthy.

The samples of blood were diluted 1:30 (20 µl of blood and 60 ml of isotonic NaCl solution buffered to pH 7.3 by veronal HCl).

Acid haemolysis was obtained by submitting the red blood cell suspension to a progressive increasing hydrogen ion concentration by dialysis against an isotonic solution of low pH (10).

The degree of haemolysis as a function of continuously reduced pH was automatically recorded by means of the Fraguograph (model D 2 Elxon Electronic Ind. Haifa, Israel).

A sample of 40 µl of the red cell suspension was introduced into a microcuvette of 0.04 ml capacity.

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classifications. According to the present classification proposed by WHO (24) the atypical case E S ought to be viewed as having a mild form of classical PKU. Diet therapy was considered necessary with regard to the present minor knowledge about the causes of mental retardation in PKU.

SUMMARY

Two infants with hyperphenylalaninemia first recognized at the age of 5 days were studied. One of the patients was a case of classical severe PKU. The other patient, on an unrestricted diet, showed only a slow rise in the plasma phenylalanine level amounting to 16 mg/100 ml at 3 weeks of age and had no phenylpyruvic aciduria. The plasma phenylalanine levels of the two infants were determined during fasting, after feeding and after intravenous phenylalanine loading. The results obtained did not reveal any significant difference in the phenylalanine turn over between the two infants.

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day of life is augmented ($p < 0.01$). The time of acid lysis which is more reduced between 1 and 3 days of life increases between 4 and 6 days of life and still augments in the second week of life. The differences are very significant ($p = 0.001$).

Subsequently the time of acid lysis shows a progressive decrease and the values are similar to the normal values after the nineteenth day of life.

The increased resistance of red cells to acid lysis and the decrease of the time of lysis increasing age are similar for the initial and for 50° and 100°.

The rate of haemolysis expressed as percent per minute is different ($p = 0.01$) only in infants aged from 2 to 3 days and from the second week of life. The rate of lysis is normal after the sixteenth day of life.

DISCUSSION

Minimal changes in pH alter the osmotic fragility of erythrocytes (19-31). The influence of pH has been attributed to changes both in the osmotic properties of haemoglobin and in the binding of cations by the haemoglobin (19).

The increase in the osmotic fragility of erythrocytes at lower pH values is the result of changes in cell water and in volume for an increase in the osmotic activity in the cell (13-27-28). The decrease of pH is associated with a relative increase in solvent water and a relative decrease in "bound water" but the mechanism whereby pH influences the amount of water involved in osmotic equilibrium is not known (27-28).

It has been suggested (12) that the change in osmotic properties of cell water at different pH levels results from changes in the effective charge on the hemoglobin molecule with dilution (28). Alterations in the relative content of Na and K and changes in temperature from 5°C to 37°C (28) does not influence the osmotic behaviour of cell water.

The present study shows that the foetal red cells are different from the adult ones with re-

Table 2 Statistical analysis (t test of Student) of time of acid lysis of red blood cells obtained in infants versus normal children

p = probability NS = not significant

Age (days)	Acid haemolysis			Rate of haemolysis
	0°	50	100	
1	$p < 0.01$	< 0.01	< 0.01	NS
2-3	$p < 0.01$	< 0.01	< 0.01	< 0.01
4-6	$p < 0.01$	< 0.01	< 0.01	NS
7-15	$p < 0.01$	< 0.01	< 0.01	< 0.01
16-30	$p < 0.01$	0.01	< 0.01	> 0.01
31-60	$p < 0.01$	< 0.01	< 0.01	< 0.01
61-90	$p < 0.01$	< 0.01	0.01	NS
91-180	p NS	NS	NS	NS
181-360	p NS	NS	NS	NS
2nd year	p NS	NS	NS	NS
1 day versus 2-3 days	p 0.01	< 0.01	0.01	< 0.01
2-3 days versus 4-6 days	$p < 0.01$	< 0.01	< 0.01	< 0.01

gard to the acid lysis and they are more resistant to the osmotic lysis when the pH of the medium decreases. This characteristic is more marked after 2 or 3 days of life and in the second week of life. The resistance to the acid lysis decreases progressively and reaches the values similar to that of the older children after 90 days of life. The normalization of the acid lysis is not related to the behaviour of the red cells to the hypotonic saline solutions (41) because the normal hypotonic lysis is reached in the second year of life (41).

The resistance to the acid lysis can be related to the amount of foetal haemoglobin that shows a gradual decline during the first weeks of life and a more rapid decline at about 2 months of age terminating in levels of about 10% at 4 months of age (11). But we have observed that the acid lysis is diminished both in red cells from thalassemia major and in the younger red cells from thalassemia trait with high and respectively low levels of haemoglobin F (42).

Moreover the loss of K of the foetal red cells does not influence the acid lysis (4-28).

The behaviour of the acid lysis is related however to the shortened life span of the fo-

Table 1 Time of acid lysis of red blood cells expressed as mean values \pm SD

Age (days)	Subjects (number)	Acid haemolysis (seconds)			Rate of haemolysis (/minute)
		0	50	100*	
1	8	181 \pm 40	238 \pm 53	416 \pm 69	49.0 \pm 14.9
2-3	11	242 \pm 36	326 \pm 37	510 \pm 43	30.8 \pm 6.2
4-6	8	190 \pm 23	272 \pm 33	450 \pm 73	43.5 \pm 13.0
7-15	10	220 \pm 33	307 \pm 27	494 \pm 52	36.5 \pm 5.7
16-30	10	189 \pm 29	274 \pm 31	453 \pm 49	41.8 \pm 4.6
31-60	9	165 \pm 19	247 \pm 21	436 \pm 80	32.9 \pm 13.0
61-90	10	151 \pm 7	227 \pm 19	374 \pm 19	43.7 \pm 9.7
91-180	16	140 \pm 17	208 \pm 23	335 \pm 74	50.2 \pm 9.3
181-360	16	138 \pm 24	216 \pm 25	330 \pm 37	47.3 \pm 12.9
2nd year	10	132 \pm 15	209 \pm 18	323 \pm 20	48.3 \pm 10.0
6-10 years	12	133 \pm 9	209 \pm 6	320 \pm 29	46.4 \pm 5.9

and of 0.5 mm path two walls of which are made of dialysing membrane. The microcuvette was held in a bath containing isotonic NaCl solution adjusted to pH 2.45 by HCl at thermostatically controlled temperature of 25°C.

The gradual increase of the number of red cells hemolysed is resulted in a progressive increase in the light transmission through the cell suspension and provides a cumulative and a derivative curve to reduced pH.

Younger cells are most sensitive to acid lysis in contrast to osmotic lysis in which the old cells are the first to lyse (10).

The results are expressed as lysis in function of time and the following measurements are:

(a) time in seconds required from the start of the curve to the initial lysis and to 50 and 100 of lysis

(b) slope of the curve between 25 and 75 of

lysis and calculation of the rate of hemolysis as per cent/minute.

Statistical analysis are determined with the calculation of the Standard Deviation (SD) and of the *t* test of Student.

RESULTS

The results of the acid lysis of red blood cells in normal children aged from 1 day to 10 years are compiled in Table 1 and in Fig. 1.

Statistical analysis were determined on the results of the infants versus the results of normal children aged from 6 to 10 years (Table 2).

The time of red cell acid lysis in the first

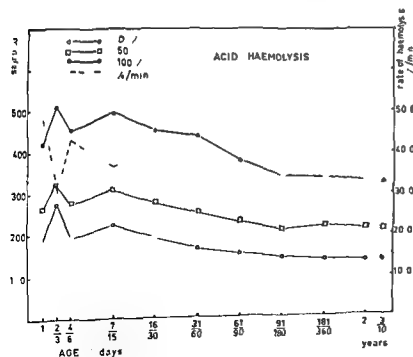


Fig. 1 Time of acid lysis of red cells (mean values) in relation to the increase of the age.

day of life is augmented ($p < 0.01$). The time of acid lysis which is more reduced between 2 and 3 days of life increases between 4 and 6 days of life and still augments in the second week of life. The differences are very significant ($p = 0.001$).

Subsequently the time of acid lysis shows a progressive decrease and the values are similar to the normal values after the nineteenth day of life.

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The behaviour of the acid lysis is related however to the shortened life span of the fo-

tal erythrocytes The life span has been demonstrated as 80 days (32) from 56 to 105 days (48) from 70 to 80 days (23) from 82 to 131 days (18), as 83 days (11) and it is very possible about 90 days

The progressive reduction of the resistance to the acid lysis is related to the disappearance of the foetal red cells and to the presence of adult type of red cells The slow diffusion of water in the foetal red cells (1) prevents the rapid increase of the red cell water when the pH falls and delays the lysis

SUMMARY

Acid lysis of foetal red cells has been investigated by means of an automated procedure with the Fragiligraph

The time of red cells acid lysis is augmented in the first weeks of life and returns normal after the ninetieth day of life

The rate of acid lysis is normal only after the sixtieth day of life

The behaviour of the acid lysis is related to the disappearance of the foetal erythrocytes and to the presence of adult type of red cells

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L ASPARAGINASE TREATMENT OF ACUTE LEUKAEMIA IN CHILDREN

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L asparaginase, an enzyme which is found in guinea pig serum, turned out to be active against certain neoplasms in rodents (4, 8) This enzyme can also be extracted from *E coli* cultures (1) Oettingen, Haskell, McElwain & Hill (6, 7, 12, 13) proved it to be effective in the treatment of leukaemia in man

The enzyme breaks down asparagine to aspartic acid cells which lack an asparagine synthesizing system can develop a lethal deficiency of this amino acid (2, 5)

It was formerly thought that in leukaemia only the asparagine dependent blasts would suffer and that other blood and bone marrow cells were not damaged but it has been shown (1, 11) that lymphocytes function normally only in the presence of asparagine, and perhaps lack a synthesizing system

Some information on the results including the course of the disease and some biochemical data are given in this article

PATIENTS AND METHODS

L asparaginase was usually given intravenously daily in a dosage of 200 U/kg bwt The injection lasted a few minutes extravasation gave little or no irritation In the first patients it was given alone but later on it was combined with prednisone 80-100 mg/m² body surface Bone marrow punctures were done at the start after 14 and 28 days of therapy The number of children treated was 18 of whom 4 were in a relapse and 14 had had no prior treatment One of them had a monocytomonoblastic type

of leukemia One formerly untreated child is discussed separately because his cell type (case 18 D K) was very unusual he is not mentioned in the table of untreated cases

Particulars are shown in Table 1 The remaining twelve were considered to have the lymphoblastic type of leukemia

In the untreated cases asparaginase therapy had to be stopped twice in case 7 because of an anaphylactic shock

In case 13 the bone marrow was severely hypocellular After cessation of asparaginase treatment prednisone was continued and the bonemarrow showed a repopulation of normal elements again

RESULTS

Bone-marrow and peripheral blood

If the definition "Complete remission" implies the presence of a normocellular bone marrow then full remissions were not so numerous (see Table 1) The hypocellularity makes it also rather illusory to say with reasonable certainty whether blastcells are present or absent An other difficulty not mentioned elsewhere was the relatively high number of lymphocytes or lymphocyte like cells often found in these bone marrow smears

In patients number 1 2 10 12 and 13 a hypocellular bone-marrow was found at the end of the treatment with signs of recovering erythro and granulopoiesis Blastcells were, with the reservations mentioned above considered to be absent As treatment was continued with cytostatics it cannot be claimed

Table 1 Patients with no prior treatment

Table 1 Patients with no prior treatment										
Patients	Sex	Age	Type of leukaemia	Duration of therapy (days)		Findings after stopping asparaginase				
				Asparaginase	Prednisone	Bone marrow		Peripheral blood		
						Cellularity	Blasts	Hb (g/100 ml)	Leucocytes (per mm ³)	Platelets (per mm ³)
1 R S	o	10	A Mo L	28	—	Hypocellular	Absent	9.5	5 600	241 000
2 M G	♀	11	A L L	28	28	Hypocellular	Absent	11.9	5 700	107 000
3 J D	♂	21	A L L	28	10	Normal	5	11.5	3 000	71 000
4 H B	♂	33	A L L	28	28	Normal	Absent	9.9	3 400	121 000
5 F L	♀	36	A L L	14	14	Normal	90	7.4	1 400	28 000
6 G M	♂	39	A L L	21	10	Normal	40*	9.8	13 600 + blasts	8 000
7 P R	♂	43	A L L	10	10	Normal	30*	9.0	2 160	10 000
8 F V	♂	410	A L L	28	28	Normal	Absent	11.6	7 600	228 000
9 M G	♀	68	A L L	28	28	Normal	Absent	14.2	4 200	139 000
10 E S	—	70	A L L	21	28	Hypocellular	Absent	12.5	5 500	101 000
11 R H	—	71	A L L	28	—	Normal	Absent	10.1	5 100	230 000
12 Y B	♂	8.2	A L L	28	28	Hypocellular	Absent	11.3	2 800	130 000
13 M H	♀	9.5	A L L	19	19	Hypocellular	Absent	11.9	1 700	40 000

that this therapy alone resulted in full remissions. A partial remission was obtained in patient 3.

In the peripheral bloodcell-counts it was found that if the marrow was hypocellular a rise toward normal numbers of erythrocytes, granulocytes and platelets was seen. However only two of the 5 patients had obtained a normal number of all the three elements. The others had subnormal numbers of one or two of the three. The platelets were more than 100 000/mm³ in 4 patients but in one they were still subnormal but within the safe range (40 000). In patients 4, 8, 9 and 11 the bone marrow was normocellular and no blastcells were detectable. The peripheral blood was normal in these four patients except for a subnormal haemoglobin content in case 4.

In the case of patient 6 asparaginase was stopped after 21 days; the bone marrow still contained more than 40% blasts and a re-commencing erythropoiesis was seen.

Patients in relapse

Four patients in relapse were treated; in two of them we believe it had no beneficial effect. Patient 14 (J J) a 14 year old boy had an acute myeloblastic leukaemia. Despite intense cytostatic drug treatment a complete remission

was not obtained after 8 months. Only a very partial remission not long lasting was the best result in this boy. Peripheral bloodmyeloblasts turned out to be very sensitive *in vitro* for asparaginase. After starting treatment the number of blasts dropped from 56 800 to 9 000/mm³ in 1 week. Thereafter there was a rise in the next 10 days.

Patient 15 (M R) was a 9 year-old girl suffering from acute myeloblastic leukaemia. She had been in a continuous remission for 13 months when a relapse developed. Various cytostatics resulted only in a very temporary partial effect. Myeloblasts from bone marrow proved to be very sensitive *in vitro* for asparaginase. The patient was treated with asparaginase 200 U/kg bwt daily for 6 days and as the number of blasts in the peripheral blood did not drop the dosage was doubled for another 7 days without success. The father wanted her to be home again and as we felt there was no real hope for a remission we could not but agree to his wish. Treatment was stopped; she died at home. An autopsy was not performed.

Patient 16 was a 10 year-old girl (M V). When she was 8 years old a diagnosis of acute leukaemia was made elsewhere and she was treated successfully. Later the initial diagnosis

Table 2 Correlation between the test of leukaemic cells for L-asparaginase requirement *in vitro* and clinical success of L-asparaginase therapy

Patients	Type of leukaemia	Percentage ³ H uridine incorporation into nucleic acids			Clinical success
		+ L asparagine	- L asparagine	+ L asparagine	
1 R S	A Mo L	100	58	31	+
5 F L	A L L	100	78	47	-
7 P R	A L L	100	60	44	+
8 F V	A L L	100	63	76	+
9 M G	A L L	100	97	78	+
10 E S	A L L	100	109	114	-
12 Y B	A L L	100	88	72	-
14 J J	A My L	100	78	32	-
15 M R	A My L	100	70	26	-
17 A T	A My L	100	99	76	+
18 D K	A Myelocgranuloc L	100	82	41	-

was doubted and all treatment was stopped. Her condition remained excellent for about 10 months when a relapse occurred. In that stage she was admitted to our hospital. Treatment was started with prednisone and asparaginase 200 U/kg bwt daily. She developed a high temperature with extreme vomiting and an extreme tenderness of the abdomen. Asparaginase treatment was discontinued. An *E. coli* sepsis was diagnosed and she died 11 days after the beginning of therapy, and 6 days after stopping asparaginase therapy. The clinical picture was suggestive for an acute pancreatitis. At autopsy however the pancreas proved to be normal. Patient 17 is an 8 year old boy with a fourth relapse of his acute myeloblastic leukaemia had become resistant to the usual cytostatics and prednisone. As our results in the other three patients with 200 U/kg bwt were not satisfying the dose in this patient was arbitrarily set at 500 U/kg bwt given intramuscularly. After 28 days a full remission was obtained.

Acute myelocytic granulocytic leukaemia

Patient 18 was a 9 year old boy (D K) suffering from an acute myelocytic granulocytic leukaemia. In culture his cells seemed to be very sensitive and he was treated with L-asparaginase alone (200 U/kg bwt). The blasts in the blood disappeared after a few days but only to

rise again a few days later. Trial with a higher dosage was not done.

Infection during treatment

Only one patient (case 16 M V) died from an infection (*E. coli* sepsis). The general impression was that infections were rarer and less severe than in our other unreported series of patients treated with rubidomycin, vincristine and prednisone.

A *staphylococcus aureus* sepsis developed in one patient (case 13 H H) during the course of treatment. She recovered with antibiotics and incision of an abscess.

In patient 1 treatment with asparaginase was started when he was still suffering from a furunculosis. Our impression was that this therapy did not interfere with the recovery from that infection.

Complications of the treatment

Allergic reactions As asparaginase is a bacterial protein it could give rise to immunological allergic reactions. They have been described e.g. by Hill et al (7). It is also possible that an allergy to this *E. coli* product exists already before the commencement of therapy. Therefore a pretreatment-test is done as a routine by injecting 10 IU intracutaneously. The reaction is read 2 hours later. In all our patients this was done and an allergic like reac-

tion was never found. None the less one patient (case 7 P R) developed an anaphylactic shock in a few minutes after completing the injection. This occurred on the tenth day of treatment. The symptoms subsided after a few hours.

Liver disorders Serum alkaline phosphatase, thymol turbidity and serum bilirubin were always normal. Slight transient rises of SGOT and SGPT were noticed. One child developed a severely disturbed liver function one week after stopping asparaginase. Total serum protein also tended to fall. There was a selective fall in the albumin level though not great enough to cause oedema.

Kidney function The slight increase of blood urea by 100–400 mg/litre was rather constant. Creatinine blood levels remained unchanged and proteinuria or urinary sediment abnormalities were not found.

Coagulation disorders In all the patients a drop of serum fibrinogen was seen. It has been demonstrated that this fall is due to decreased synthesis of fibrinogen (3). Other coagulation factors were not investigated. We cannot state that more haemorrhages occurred than with other forms of treatment.

Uric acid metabolism Blood levels remained normal; the urinary excretion was initially high but dropped to normal values during treatment. Allopurinol was not used; renal colic or other signs of stone formation was not observed.

Further toxicity Gastrointestinal symptoms were slight or absent; neurological toxicity was not observed. In our opinion this drug is usually well tolerated.

Correlation between in vitro test and clinical effectiveness

The test for L-asparagine requirement was performed with cell suspensions from the bone marrow or from the buffy coat (13). This assay involves measuring the incorporation of ^3H uridine into nucleic acids by cells incubated in the presence and absence of L-asparagine and in the presence of L-asparaginase respec-

tively. In Table 2 for 11 from 18 patients described above the correlation is given between the L-asparagine dependency of the leukaemic cells *in vitro* and the response of the patients to L-asparaginase.

The results given in Table 2 show that in 6 out of 11 cases (patients 9, 10, 14, 15, 17, 18) there was a reverse correlation between *in vitro* results and the effect of clinical treatment.

CONCLUSION

L-asparaginase may be used for the initial treatment of acute leukaemia. The remission rate after four weeks of treatment does not seem to be higher than that obtained with combinations of cytostatics and prednisone. If looked for, asparaginase gives rise to many biochemical abnormalities which turn out to be reversible. Fibrinogenopenia ought to be a real disadvantage but in our experience we felt it did not cause many difficulties.

L-asparaginase is usually well tolerated. Life-threatening allergic manifestations may however occur rather unexpectedly.

The results in the four children with relapse were disappointing though three of them were of the myeloblastic type which is less sensitive to most types of therapy than the lymphoblastic type.

The value of the *in vitro* asparaginase sensitivity test is poor; a hopeful outcome may be followed by an *in vivo* failure or vice versa. The dosage of 200 U/kg bwt seemed to be very satisfying. If the blast cells are not asparagine-dependent or if they can easily develop the asparagine synthesizing system, a higher dosage scheme would not work either. Therefore we doubt if the very high dosages used by others (10, 12) are a real advantage. Toxicity surely increases with increased dosage; effectiveness is another question.

If our results are compared with Mathe et al (10) the remission rate is higher in this series of patients. We presume that this was due to the combination treatment with prednisone because their patients were treated with L-aspara-

ginase only but in much higher dosage 400 to 800 U/kg bwt daily in 2 divided doses. On the other hand McElwain & Hardisty (12) gave 1 000 U/kg bwt but alone, to 9 children with acute lymphoblastic leukaemia in relapse. After pretreatment with cytosine arabinoside, the blasts in the bone-marrow varied from 99 to 11%. This was followed immediately with asparaginase only for 14 to 28 days. Full remissions were obtained in 8 out of 9 patients.

SUMMARY

The results of asparaginase prednisone therapy in children are reported. In 12 children with untreated acute lymphoblastic leukaemia 9 remissions were obtained, there were 2 failures and in one patient therapy had to be stopped because of an anaphylactic shock.

One child with an acute myelocytic granulocytic type of acute leukaemia did not respond. One child with an acute monocytic monoblastic leukaemia was given asparaginase only. He responded very well.

Four patients with a relapse of an acute myeloblastic leukaemia were treated; there were two failures: one child died too early from an infection to be evaluated. One child showed a very good reaction. Serious side effects were few, the drugs gave many biochemical disturbances including a constant hypofibrinogenemia but these were well tolerated and reversible.

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SEPARATION BY GEL FILTRATION AND MICRODETERMINATION OF UNBOUND BILIRUBIN

1 *In Vitro* Albumin and Acidosis Effects on Albumin Bilirubin Binding

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It is now possible to separate unbound bilirubin from serum either by dialysis (16) CaCO_3 adsorption (25) or gel filtration (5, 8, 9, 10, 27). As the unbound bilirubin serum concentration is low we adapted in our study an extraction technique on Sephadex micro-columns and a diazoreaction method in order to analyze the small serum volumes usually drawn from the newborn infants.

Albumin and pH effect on albumin bilirubin binding has been studied on newborn sera as well as on commercial bilirubin and human albumin mixtures.

MATERIAL AND METHODS

Pediatric Versatol (Warner-Chilcott Laboratories)

Bilirubin (Calbiochem) dissolved in Na_2CO_3 0.1 M just before use. Human albumin (Centre National de Transfusion Sanguine—Paris) 78% purity dissolved in PO buffer 0.1 M.

Sephadex G 75 Medium (Pharmacia Uppsala Sweden) suspended in PO buffer 0.1 M.

Serum from icteric premature or full term infants submitted to exchange transfusion for erythroblastosis foetalis.

Manipulations take place at room temperature ($20-24^\circ\text{C}$) in half light. A fine catheter closed with a grip is adjusted to the lower extremity of 5 ml pipettes (0.6 cm diameter, 0.1 ml graduations) cut at 70 cm height so that any volume added with Pasteur pipet

Abbreviations: PAG polyacrylamide gel; PSP phenolsulfonphthalein dye; HIBABA 2-(4-hydroxybenzeneazo) benzoic acid. In this study unbound bilirubin is assumed to be unconjugated.

tes on the column or collected in analysis tubes can be directly appreciated from the column graduations. In order to lessen the dilution of the samples on the column the Sephadex bed fills only 2.3 ml on the column graduations. The 0.5 ml sample is layered over the Sephadex. The flow is stopped and the walls are washed with 0.3 ml PO buffer 0.1 M. When a thin layer just covers the Sephadex bed the outlet is closed and 1.5 ml PO buffer is added and the first 1.5 ml of eluant is collected (Peak 1" containing all the proteins of the sample and all bilirubin bound to albumin). The column is washed with 2 ml buffer. In order to trap all unbound bilirubin left on the Sephadex 0.5 ml albumin solution (10 mg/ml in PO buffer 0.1 M, pH 7.4) is added and allowed to penetrate almost completely into the Sephadex. The flow is stopped and the column is filled with eluant buffer. 30 min later the outlet is opened again after 0.3 ml buffer has drained into the bed. 1 ml eluant is collected (Peak 2" containing all human albumin added and bilirubin previously left on the column).

When washed with 5 to 10 ml buffer the same column can be used repeatedly. A well trained person can perform separation and bilirubin determination of one serum within one hour. If one works with 4 columns it is possible to analyze 4 samples in 2 hours.

For the unbound bilirubin determination the Michaelsson technique (18) sensitivity has been increased by concentrating several reagents in order to obtain a coloration of adequate intensity in a reduced volume.

Sample	0.25 ml
Diphylla	0.20 ml (unmodified reagent)
Diazoreagents	0.10 ml (2.5 × concentrated reagent)
Ascorbic acid	0.01 ml (5 × concentrated reagent)
Fehling	0.15 ml (unmodified reagent)
Final volume	0.71 ml

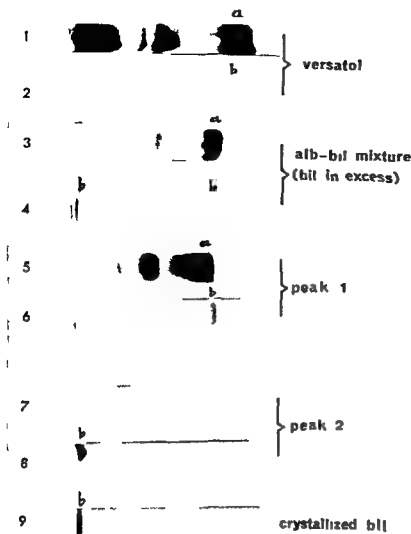


Fig 1 Identification of bound and unbound bilirubin by PAG electrophoresis. Protein bands move from left to right and are located with 1% (w/v) Amido black in 7% (v/v) acetic acid (gels 1, 3, 5, 7). Yellow bilirubin spots are visible on unstained gels (gels 2, 4, 6, 8 and 9). a = albumin, b = bilirubin, peaks 1 and 2 from Sephadex column. In this particular case, peak 2 = not extracted with an albumin solution but with Na₂CO₃ 0.1 M vs crystallized bilirubin is dissolved in Na₂CO₃ 0.1 M.

Reading is done at 600 μ m on a Jobin Yvon spectrophotometer in pyrex microcuvettes (25 \times 10 \times 2 mm).

Pediatric Versatol is used for the standard curve; the assay was linear within the 0.1 to 2.0 μ g interval.

Two different amounts of bilirubin assayed in the same conditions gave respectively 0.79 μ g \pm 0.06 μ g and 1.73 μ g \pm 0.06 μ g (mean \pm 1 SD, $n=10$ in each case).

RESULTS

Gel filtration. Separation on Sephadex column chromatography and identification of both bilirubin types are shown on Fig 1. On PAG electrophoresis (12) pure bilirubin or unbound bilirubin stays at the origin whereas bound bilirubin migrates with albumin.

In experiments using either pure bilirubin solution, serum or albumin-bilirubin mixtures containing one or both bilirubin types, the total

bilirubin recovered after gel filtration was 97.7% \pm 2.9%, $n=14$ (mean \pm 1 SD).

In the same conditions each of 10 samples were analysed on two different columns and the difference observed between each column in terms of total bilirubin recovered was 1.35% \pm 2.05% (mean \pm 1 SD).

pH and albumin concentration effects. A constant bilirubin amount is added on columns conditioned at different pHs. The column is loaded with an excess albumin solution (pH identical to the pH of the column) in order to pick up bilirubin.

The relation existing between bilirubin attachment and pH could be apparent and is actually due to a decrease of bilirubin solubility in water when the pH is lowered (1). As a matter of fact when albumin and bilirubin are mixed in identical conditions of pH and

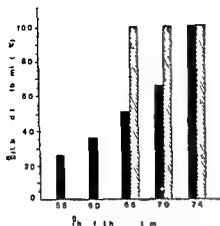


Fig 2 Bilirubin bound to albumin at different pHs. Sephadex G 25 columns are conditioned with PO buffer 0.1 M at pHs 5.5, 6.0, 6.6, 7.0 and 7.4. After addition of 25 μ g bilirubin (dissolved in Na_2CO_3 0.1 M) the columns are washed with the corresponding buffer. Albumin (125 mg) dissolved in PO buffer 0.1 M (pH identical to the pH of the column) is added on the column. Bound bilirubin (peak 1') is measured and expressed as % of the total bilirubin added on the column when the binding takes place on the column (dark areas) or in the test tube before gel filtration (shaded areas).

concentration and then added on the column. Bilirubin is completely bound to albumin whatever the pH might be (Fig 2).

In order to avoid these solubility problems on the column at low pHs, albumin and bilirubin are at first mixed in variable proportions and at different pHs (Fig 3).

Bilirubin binding becomes higher and higher as albumin proportions increase in the mixtures. Furthermore, for a given albumin bilirubin mixture, the proportion of protein bound bilirubin increases with the pH.

Acidification of icteric sera. Only protein bound bilirubin was detected in 4 icteric sera. After acidification with PO₄ buffer 0.1 M pH 5.0, unbound bilirubin appeared and the proportion of this fraction seemed to be related to the degree of acidosis (Table 1).

Role of the pH of the column. As pH modifies the relative proportions of bound and unbound bilirubin, it was important to find out whether the pH of the column should be adjusted each time to that of the serum before being analysed.

As seen in Table 2, the equilibrium existing in a sample between both bilirubin fractions is not affected by the pH of the column (at least between pH 7.0 and 7.4). Therefore, we use columns systematically conditioned at pH 7.4 for the analysis of newborn infants sera.

DISCUSSION

An equilibrium exists in the plasma between the non conjugated unbound bilirubin fraction and the protein bound bilirubin fraction. Plasma concentration of unbound bilirubin may represent 1% of the total bilirubin (20) and depends on the serum protein amount (more precisely albumin amount) and on its bilirubin binding capacity (3, 10, 17, 20, 27, 30) on the acido-basic equilibrium (2, 3, 13, 14, 15, 16, 19, 28, 33) and on endogenic or exogenic competitive compounds which occupy binding sites on albumin (4, 6, 11, 17, 23, 29).

It is interesting to know whether in a given serum albumin is still capable of binding additional bilirubin or is already saturated (7, 17, 20, 24, 26, 31). If unbound bilirubin is toxic, it seems however more logical to isolate and determine this fraction and to study

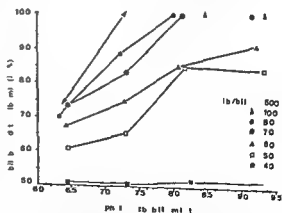


Fig 3 pH effect on bilirubin binding capacity of albumin. Bilirubin (dissolved in Na_2CO_3 0.1 M) is mixed with albumin solution in PO buffer. Albumin bilirubin weight ratios as indicated. The pH of albumin solutions is chosen according to the final pH desired in the albumin bilirubin mixtures. Columns are adjusted to this final pH with PO 0.1 M buffer. 25 μ g bilirubin are added on the column and bound bilirubin is measured in peak 1'.

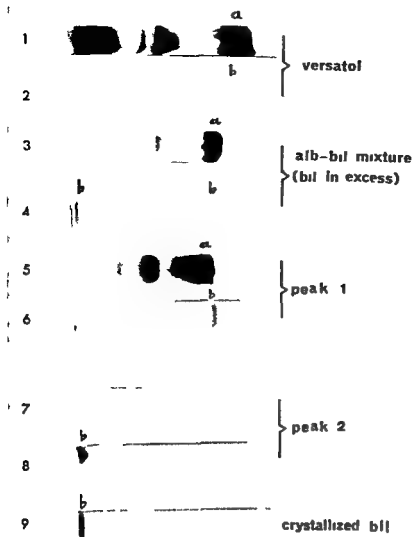


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The relation existing between bilirubin attachment and pH could be apparent and is actually due to a decrease of bilirubin solubility in water when the pH is lowered (1). As a matter of fact, when albumin and bilirubin are mixed in identical conditions of pH and

most serum compounds and competitive endogenous or exogenous substances. The effect of such substances as fatty acids α globulins lipoproteins on albumin bilirubin binding has not been thoroughly studied (25-32) and these factors could account for the individual variations in the albumin binding capacity observed with different sera (25).

SUMMARY

A gel filtration technique and a diazoreaction method for unbound bilirubin separation and determination directly applicable to small blood volumes has been described.

Precision of the microdetermination reproducibility of the separation method and bilirubin recovery have been very satisfactory in our hands.

The effects of pH and relative albumin concentration on the albumin bilirubin binding were studied in sera and in commercial albumin bilirubin mixtures.

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Table 1 *Unbound bilirubin appearance in acidified icteric sera^a*

Before acidification				After acidification			
Total bil conc in serum ($\mu\text{g/ml}$)	pH	Bil added on column (μg)	Unbound bil recovered (μg)	pH	Bil added on column (μg)	Unbound bil recovered (μg)	Unbound bil recovered (of total bil)
180	8.94	90	0	6.04	26	12	4.6
180	8.94	90	0	6.37	40	16	4.0
200	8.55	100	0	6.88	66.6	12	1.8
160	8.65	80	0	7.06	61.5	10	1.5

^a Sera previously stored in refrigerator are analysed on Sephadex columns before and after acidification with PO_4 buffer 0.1 M pH 5.0. Bilirubin is determined in serum and in peak 2 before and after acidification.

the factors involved in its plasma concentration.

With the Rutkowski's technique (25) one can separate unbound from the protein bound bilirubin, unfortunately, we could not recover the unbound bilirubin when it was adsorbed on CaCO_3 .

Sephadex gel filtration has been used by previous authors (5, 8, 9, 10, 27) and we found this technique to be adaptable for newborn icteric serum analysis with good reproducibility and bilirubin recovery.

Acidosis increases unbound bilirubin concentration in serum and tissues (20). With sera and albumin bilirubin mixtures we observed that acidosis not only decreases the binding of bilirubin by albumin but also unbinds bilirubin already attached to albumin in a pro-

portion which seems to be in relation to the degree of acidosis.

On the other hand it is possible to compensate for the acidosis effect by increasing the albumin concentration. These *in vitro* experiments once more emphasize the importance of acidosis in kernicterus production and the protective effect of albumin.

The number of bilirubin molecules which can be bound by one albumin molecule has been determined by several means with various results (9, 10, 16, 21, 25, 27).

Furthermore using the same technique the albumin bilirubin molar ratio varies according to whether one is working with crystallized or purified albumin or with serum (10, 22, 25).

With Sephadex gel filtration at pH 7.3, we found that 78 mg of albumin is necessary to bind 1 mg of bilirubin.

When albumin and bilirubin molecular weights are chosen as 69 000 and 585 respectively the calculation shows that *in vitro* 1 albumin molecule binds 1.5 bilirubin molecule in our experimental conditions. This observation is consistent with the results with Sephadex obtained by Keenan et al (10). With the same technique Keenan et al (10) and Kaufman et al (9) working on sera found that 1 albumin molecule binds 1 bilirubin molecule.

Albumin purification steps may have modified the binding capacity of albumin although its electrophoretic and immunologic properties are identical to that of albumin in serum. This commercial albumin is probably cleared out of

Table 2 *Effect of the initial pH of the column on the equilibrium between unbound and bound bilirubin^a*

	Peak 1		Peak 2	
pH of the buffer on the column	7.0	7.4	7.0	7.4
Alb/bil weight ratio = 80				
Final pH of the mixture 7.0	88.8	88.1	11.2	11.9
Alb/bil weight ratio = 50				
Final pH of the mixture 7.4	69.3	68.6	30.7	31.4

^a Bilirubin in Na_2CO_3 is mixed to albumin (dissolved in PO_4 buffer 0.1 M pH 6.6) 0.5 ml of each mixture is layered on columns conditioned respectively at pH 7.0 and 7.4. Bilirubin from peaks 1 and 2 is measured and expressed as % of the total bilirubin recovered.

SEPARATION BY GEL FILTRATION AND MICRODETERMINATION OF UNBOUND BILIRUBIN

II Study of Sera in Icteric Newborn Infants

P ZAMET and F CHUNGA

From the Centre de Recherches Biologiques Neonatales Paris France

Numerous studies *in vitro* and *in vivo* have established that bilirubin cytotoxicity involved in kernicterus from neonatal jaundice is related to the only ultrafilterable fraction not albumin bound (19-20). This unbound bilirubin can be extracted from cerebral tissues after kernicterus in man as well as in animals (3-4, 7-29); it impairs the cellular functions (4) chiefly through a prevalent uncoupling effect blocking the oxidative phosphorylation (2, 4, 17-26, 31).

The absence of toxicity of the protein bound bilirubin has been suggested by its exclusively extra-cellular distribution and is now well established (2, 4, 17-18). For these reasons separation of the free bilirubin and determination of its serum level has been of great interest. Technical difficulties related to the low levels of unconjugated bilirubin that may be present in jaundice sera have been solved. Separation can be precisely performed through gel filtration with Sephadex (1, 8, 9, 10). Recently this method allowed small quantities of free bilirubin to be extracted and accurately measured (1).

The purpose of the present study was to demonstrate the possible presence of unbound bilirubin during neonatal jaundice, to measure its levels and to study whether any correlation could be shown in relation to total bilirubinemia.

MATERIAL AND METHODS

45 blood samples were obtained from 35 jaundiced newborns.

23 samples were taken from infants with erythroblastosis fetalis (Rhesus or ABO incompatibility—Table 1) and 22 from premature infants (Table 2). Most measurements were carried out on blood obtained by the first increment at the start of exchange transfusion. In some cases a second sample was taken at the end of the exchange procedure.

Two infants had symptoms of kernicterus; 5 autopsies were performed.

Repeated serum assessments of total bilirubin (Fig. 2), free bilirubin, free fatty acids, total protein levels and of albumin were carried out after infusion of lipid emulsion (2 g/kg) and amino acids solution¹ (0.127 g nitrogen/kg) in a jaundiced infant. This infant had a gestational age of 35 weeks and had multiple malformations potentially causing death but no respiratory distress and no cardiac failure (unfortunately no autopsy was performed).

Total bilirubin determinations were carried out by the Michaelis technique (16). Free bilirubin was separated by gel filtration with Sephadex then measured with the Chunga-Lardinois micromethod (1). Total serum protein content was determined by the biuret method after polyacrylamide electrophoresis of a serum sample; the albumin fraction was evaluated on a spectrophotometer recorder. Free fatty acids were assessed with the Laurell's micromethod (11).

RESULTS

General results

Among the 45 samples obtained just before exchange transfusion or in the course of jaundice 25 had noticeable amounts of free bilirubin.

¹ Intralipide 20% and Aminosol 100 Vitrum.

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Table 1 *Newborns with Rhesus or A B O incompatibility Pre and Post exchange-transfusion (E T) samples*

Case	Sex	Term (weeks)	Birth weight (g)	Hours of life	Total Bilirubin mg/100 ml		Unbound Bilirubin mg/100 ml	
					Pre E T	Post E T	Pre E T	Post E T
(a) Newborns with Rhesus Incompatibility								
1 Yad	♂	36	1 940	4	9.5	2.4	0.04	0.00
2 Bru	♂	30	1 950	9	19.0	8.3	0.24	0.08
3 Lau	♀	38	3 080	70	28.0	5.3	0.36	0.20
4 Lou	♀	38	3 720	43	15.0	6.2	0.16	0.32
5 Ber	♂	37	3 330	57	18.0	8.2	0.00	0.00
6 Mez	♂	40	3 330	62	16.0		0.00	
7 Dum	♀	36	2 750	24	14.8		0.16	
8 Bou	♂	39	2 750	44	18.8		0.05	
9 Vei	♀		2 300	59	21.2		0.20	
10 Lon	♀	41	3 230	29	17.2		0.00	
11 Lon	♀	41	3 230	47	14.2		0.08	
12 Don	♂	38	3 700	22	18.0		0.00	
13 Bri	♀	41	2 780	86	27.0		0.00	
14 Bra	♂	41	2 300	86	38.0		0.56	
15 Adr	♂	41	3 000	99	35.2		1.00	
(b) Newborns with A B O Incompatibility								
1 Mat	♂	41	3 340	55	23.8	6.4	0.12	0.00
2 Bur	♂	38	3 660	79	23.0	6.3	0.16	0.00
3 Pla	♂	38	3 400	91	23.5	6.0	0.08	0.00
4 Tal	♂	41	3 100	61	19.5	6.8	0.16	0.00
5 Dra	♂	41	3 250	69	22.2	8.1	0.16	0.00
6 Que	♀	41	3 960	60	27.6		0.12	
7 Que	♀	41	3 960	62	19.0		0.00	
8 Rac	♀	38	2 280	73	30.0		0.36	

rubin (0.1 mg/100 ml or more). Levels ranged from 0.1 mg to 1 mg/100 ml, but in only five cases (3 erythroblastosis and 2 prematures) the level exceeded 0.3 mg/100 ml. Levels in excess of 0.5 mg/100 ml were exceptional in this series, only two cases were observed and both had erythroblastosis fetalis. These infants were admitted too late at the special care unit and their total bilirubin levels were above 35 mg/100 ml.

Among the 25 samples with noticeable free bilirubin levels 12 had a total bilirubin of 20 mg/100 ml or more, 8 infants (all prematures) had total bilirubin levels less than 18 mg/100 ml, one of the latter had a fairly high amount of free bilirubin (0.35 mg/100 ml) with a total bilirubin of 17.8 mg/100 ml, another had a significant level of free bilirubin (0.21 mg/100 ml) with a total bilirubin of 12 mg/100 ml.

Generally speaking there is no correlation between the amount of unbound bilirubin and the total bilirubin (Fig. 1).

Except for one case, all the free bilirubin levels after exchange transfusion were much lower. Often the unbound fraction had completely disappeared.

Individual cases

(a) In three cases a kernicterus was observed. Two infants had clinical symptoms, both were late admissions with extremely high levels of total bilirubin.

In one case (sample number 14, Table 1) total bilirubin was up to 38 mg/100 ml and free bilirubin level was 0.56 mg/100 ml. This infant had a twin whose total bilirubin was 27 mg/100 ml without any detectable free bilirubin; the twin did not show symptoms of kernicterus (sample number 13, Table 1).

In the second case total bilirubin was 35.2 mg/100 ml and free bilirubin was 1 mg/100 ml.

A case of kernicterus occurred in a premature infant of 28 weeks gestational age with

Table 2 Icteric premature infants without incompatibility

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Case	Sex	Term (weeks)	Birth weight (g)	Hours (H) or days (D) of life	Total Bilirubin mg/100 ml		Unbound Bilirubin mg/100 ml	
					Pre E.T	Post E.T	Pre E.T	Post E.T
a) Pre and post Exchange Transfusion (ET) samples								
1 Poi	♂	43	2 450	H 63½	21.5	4.7	0.20	0.00
2 Pig	♂	33	1 940	D 6	20.3	2.6	0.12	0.00
3 Zer	♂	36	2 400	D 4½	16.4		0.16	0.04
4 Leb	♀	34	1 600	D 4	20.0	8.9	0.16	0.00
5 Ody	♀	32	1 500	H 72	20.2	6.6	0.24	0.06
6 Ody	♀	32	1 500	H 81	19.1	7.8	0.22	0.00
7 Fic	♂	36	3 200	D 5½	17.2	5.2	0.00	0.00
b) Sample during the course of hyperbilirubinemia								
8 Fic	♂	36	3 200	D 6	11.4		0.00	
9 Leb	♀	34	1 600	D 4	20.0		0.05	
10 Cas	♂	37	2 800	H 82	12.0		0.21	
11 Rey	♂	28	1 000	D 5½	15.4		0.16	
12 Rey	♂	28	1 000	D 6	15.2		0.16	
13 Ang	♂	30	2 210	D 5	15.8		0.00	
14 Pig	♂	33	1 940	D 6½	16.6		0.00	
15 Zer	♂	36	2 580	D 5½	18.2		0.00	
16 Bai	♀		1 270	H 74	15.5		0.10	
17 Pat	♂	37	2 650	D 4½	17.8		0.35	
18 Pat	♂	37	2 650	D 5	17.4		0.16	
19 Lep	♀	36	2 370	D 4	18.6		0.00	
20 Lep	♀	36	2 370	D 6	17.6		0.00	
21 Lep	♀	36	2 370	D 6½	17.9		0.00	
22 Deb		34	1 700	D 3½	14.2		0.10	

a birth weight of 1 000 g two samples showed an unbound bilirubin level of 0.16 mg/100 ml while total bilirubin was 15.4 and 15.2 mg/100 ml respectively. This infant had no respiratory distress, acid base balance was correct except in the last six hours before death (pH 7.03 P_{aCO_2} 56 mmHg BE -17 mEq P_{aO_2} 57 mmHg). At the autopsy a small hemorrhage was noted in the germinative zone. Kernicterus was evident with yellow pigments in habenula in the third cranial nerve nucleus and in the nuclei situated under the floor of the fourth ventricle.

(b) Four additional autopsies were performed on infants who had presented free bilirubin levels ranging from 0.1 to 0.24 mg/100 ml. None of them showed any sign of kernicterus. Three were prematures (birth weight 1 500 g, 1 270 g, 1 950 g) and they had an intraventricular hemorrhage.

(c) The consequence of infusing a lipid emulsion (essentially free fatty acids) and amino acids solution is presented in Fig 2.

At the beginning of the infusion total bilirubin was 14.2 mg/100 ml and only an insignificant amount of unconjugated bilirubin could be detected (0.08/100 ml). Half an hour after the infusion an important rise of the free bilirubin level was observed (0.44 mg/100 ml) followed by a slow decrease. The initial level was reached after 96 hours. During this period an isolated infusion of amino acids did not modify the decrease rate.

When the total bilirubin was 5.7 mg/100 ml with no free bilirubin infusion of lipids and amino acids did not trigger any appearance of free bilirubin.

COMMENTS

The most striking finding is that no correlation can be established between the total bilirubin level and the amount of unbound bilirubin (Fig 1) neither in infants with erythroblastosis fetalis nor in prematures (Tables 1 and 2).

Table 1 Newborns with Rhesus or A B O incompatibility. Pre and Post exchange transfusion (E T) samples

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other hand the presence of unbound bilirubin even in fairly high levels does not necessarily result in lesions of central gray nuclei (samples 5 6 10 16 Table 2 2 Table 1) Finally when there is no free bilirubin there will be no risk of kernicterus This is suggested by experimental studies (2 4 17 18) and the case history of the twins in which one had kernicterus with high total bilirubin and high free bilirubin while the other free of symptoms was in spite of a high level of total bilirubin together with an insignificant amount of free bilirubin

These data point out that the presence of unbound bilirubin only represents an index of risk other factors certainly play a role in producing cerebral lesions at a given level of free bilirubin The status of the blood brain barrier (or perhaps the permeability of cell membrane by itself) may undergo certain changes through anoxia or hypercapnia (12)

It is usually accepted that gestational age plays a part in the degree of permeability of the blood brain barrier It should however be noticed that some authors argue against the immaturity of the blood brain barrier in any newborn during the first days of life (4 27) The individual varying sensitivity and chiefly the duration of exposure of brain tissues to unbound bilirubin (in relation to the promptitude of exchange transfusion) are likely to influence the development of kernicterus

Larger series are of course necessary to establish correlations between the free bilirubin level (with regard to the exact duration of this plasmatic level) and the occurrence of kernicterus as assessed by pathology or by careful clinical evaluation after a long follow up

SUMMARY

Extraction of unbound bilirubin by Sephadex gel filtration and measurement by a micro-method were carried out in 45 sera from jaundiced newborns (hemolytic disease or prematures) In about half the cases some free bilirubin with values in the range 0.1 to 1 mg/100

ml was detected Levels above 0.5 mg/100 ml were exceptional (only 2 cases) Five autopsies were performed in prematures with unbound bilirubin levels above 0.1 mg/100 ml and one kernicterus was found Two infants with unbound bilirubin levels of more than 0.5 mg/100 ml had clinical symptoms of kernicterus No symptoms of kernicterus were observed in infants with high total bilirubin together with an insignificant amount of unbound bilirubin

The main interest of this method lies in the direct assessment of a toxic element originating from varying bilirubin and albumin levels and the micro-environment of the bilirubin albumin binding

But other factors such as the cell membrane permeability and/or the duration of exposure are likely to play a prominent role

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr P. non Dr Poulain and their staff (Service des ictères du nouveau-né Centre Départemental de Transfusion de Paris) for having kindly supplied the pre and post exchange transfusion samples and to Dr M. Couchard for the English translation of our initial manuscript

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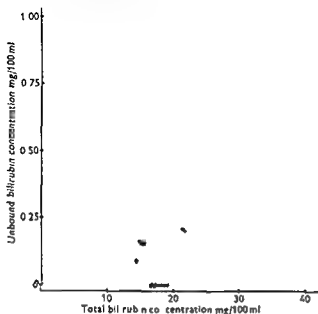


Fig 1 Relationship between total and unbound bilirubin

This absence of correlation might be expected as long as the bilirubin albumin binding is known to depend upon the levels of bilirubin and albumin, and on the environmental conditions of acidbase balance (12, 13, 21). It depends also on other factors such as serum lipoproteins (30) and substances competing with bilirubin at the albumin site, for example bile salts (5), free fatty acids (7, 22, 24), hematin (20) and various anionic exogenous compounds (7, 8, 14, 15, 20, 23).

The competitive action of free fatty acids is shown by the almost experimental case

history on Fig 2. Consequently, the intravenous infusion of lipid emulsions is a quite dangerous method in jaundiced newborn; it should not be employed unless the level of the total bilirubin is low.

The absence of relation between total bilirubin and unbound bilirubin can be compared with the results of Odell (23). This author found no correlation between the saturation of the serum albumin and the concentration ratio of the bilirubin to total protein of the serum in infants with erythroblastosis fetalis. However, a linear correlation was found between these two parameters in non hemolytic jaundices.

All techniques dealing with the assessment of residual capacity of fixation for bilirubin by serum proteins (PSP, HBABA, or salicylate (15, 23, 25, 29)) have the drawback of being indirect methods.

The advantage of free bilirubin measurement is that the amount of this toxic fraction results from a balance between bilirubin and albumin in a variable microenvironment.

The small number of autopsies (5) and kernicterus (2) in this series is inadequate to prove any correlation between the presence of free bilirubin and kernicterus. However, these preliminary data suggest that kernicterus can be observed in spite of low levels of total bilirubin (samples 11 and 12, Table 2). On the

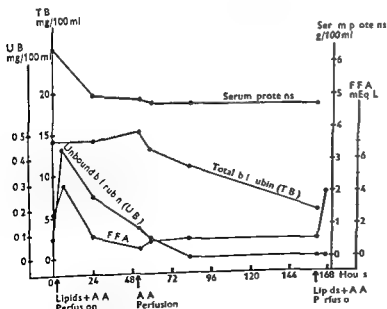


Fig 2 Variations of total and unbound bilirubin, FFA, total proteins under lipids and amino acids perfusion (albumin accounts for 50-53% of total proteins)

A FOLLOW UP STUDY OF PHYSICAL GROWTH FOLLOWING FAILURE TO THRIVE WITH SPECIAL REFERENCE TO A CRITICAL PERIOD IN THE FIRST YEAR OF LIFE

E E EID

From the Department of Child Health Sheffield University Sheffield England

It has been suggested that if a child suffered from some condition in the first year which slowed his normal rapid growth the child might not achieve his full growth potential after correction of that condition (14). There has been a dearth of studies on the subsequent physical growth of children who had failure to thrive associated with major surgical intervention in the first year of life. Similarly there has been a lack of documented studies about the existence of a postnatal critical period for physical growth caused by medical or surgical illnesses during the very early part of the first year.

Poor nutrition during the suckling period in the rat followed by realimentation reduced the eventual size of the adult rat (16).

It was reported (3, 9, 29) that children who had failure to thrive had subsequent normal physical growth while others (1, 2, 7) found that more than one third of the children had subsequent growth failure. The retarding effect of early malnutrition and undernutrition on subsequent physical growth has been reported (8, 22). The subsequent weight of children who had undergone neonatal surgical procedures was reported to be within normal limits (5, 9) or lighter than normal (6)

using Tanner's charts. A low birth child (birth weight below 2500 g) was included if his weight fell progressively below the 3rd percentile.

Children were chosen after reviewing the index cards, medical records and theatre books as far back as 1959 under the following diagnoses: idiopathic failure to thrive, feeding problems, steatorrhea, coeliac disease, fibrocystic disease of the pancreas, idiopathic hypercalcaemia, disaccharidase deficiency, renal acidosis, nephrogenic diabetes insipidus, congenital heart disease, mental retardation (except mongols and cerebral palsy), oesophageal atresia, Hirschsprung's disease, intestinal obstruction, duodenal atresia, exomphalos and patent ductus arteriosus (PDA) ligated in and after the first year (up to 8 years).

151 children were considered to have had failure to thrive. Beside these another 14 cases were chosen whose PDA was ligated after the first year and who were below the 10th percentile in weight before the operation. They were traced by a letter requesting the parents to bring the child to the Children's Hospital, Sheffield. The consultant paediatricians were requested to examine children who were living far away. Out of 165 cases data on 132 patients were obtained i.e. 74 response and of these 25 patients were examined by consultant paediatricians. These 132 cases were placed in three groups.

Treated group includes 77 cases in whom the cause of failure to thrive was corrected completely with the exception of PDA cases ligated after the first year.

Untreated group includes 35 cases in whom the cause was or could not be corrected.

PDA group ligated after the 1st year includes 10 cases.

B Control samples

Control group includes 75 healthy children from the fracture clinic.

Siblings group includes 24 children. 12 were siblings of children in the treated group, 8 were siblings of children in the untreated group and 4 were siblings

CLINICAL MATERIAL AND METHODS

A Failure to thrive samples

Children were included in the study if their weight during the first year fell below the 3rd percentile

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children aged 5 years and over. For children below 5 years Hammond's (11) tables were used.

Head circumference. Standard scores were calculated from Westroff & Barber (28) tables for children aged up to 8 years and Watson & Lowrey (27) tables for children older than this.

Triceps and Subscapular subcutaneous folds. Percentiles were obtained by plotting the measurements on Tanner & Whitehouse (24) charts.

Chest circumference. The Harvard and Iowa percentile tables published in Nelson Textbook of Pediatrics (1964) were used to obtain percentile groups.

Skeletal age. The standards of the Greulich & Pyle Atlas (10) as well as Stuart's tables published in this Atlas were used to calculate the standard scores of skeletal age.

To homogenize the differences due to age and sex the measurements were converted into percentile groups and/or standard scores. The standard scores were calculated using the following equation: standard score = $(X - \bar{X})/SD$ where X is the measurement, \bar{X} is the mean for the age and sex and SD is the standard deviation of the mean.

The decimal age was used throughout (25).

RESULTS

A. Using the percentile distribution

In Table 2 the total number and percentage of children who were below the 3rd percentile in height and in weight in each group and diagnostic category were shown.

The percentile distribution of the control group for height and for weight did not differ significantly from that of the normal population as represented by Tanner's distribution (using chi square test). In the treated group 16 lay below the 3rd percentile in both height and weight in the surgically treated cases.

Table 3 Triceps subcutaneous fold percentile distribution

Percentile groups		<10	10-50	>50
Control (n=77)	n		11	61
			15	85
Treated ^a (n=59)	n	10	22	27
		17	37	46
Untreated ^b (n=30)	n	7	11	12
		23	37	40

^a χ^2 (control and treated) = 5.68 $p < 0.001$

^b χ^2 (control and untreated) = 24.70 $p < 0.001$

Table 4 Subscapular subcutaneous fold percentile distribution

Percentile groups		<10	10-50	>50
Control (n=72)	n	12	31	29
		17	43	40
Treated (n=59)	n	16	29	13
		28	50	22
Untreated ^b (n=30)	n	8	16	6
		27	53	20

^a χ^2 (control and treated) = 4.45 $p < 0.20$

^b χ^2 (control and untreated) = 4.73 $p < 0.10$

Table 5 Chest circumference percentile distribution

Percentile group		<10	10-50	>50
Control (n=75)	n	6	15	54
		8	20	72
Treated (n=58)	n	14	20	29
		24	36	41
Untreated ^b (n=35)	n	11	7	11
		38	24	38

χ^2 (control and treated) = 13.12 $p < 0.01$

^b χ^2 (control and untreated) = 15.25 $p < 0.001$

26% in height and 22% in weight were below the 3rd percentile while in the medically treated cases 21% in height and 42% in weight were below the 3rd percentile. In the untreated group 37% lay below the 3rd percentile in both height and weight.

In the treated group of the 19 children who were below the 3rd percentile in height at follow up 8 cases had completed their surgical treatment in the first year. 3 cases of oesophageal atresia had primary anastomosis early in the first year followed by serial oesophageal dilation. 3 cases of oesophageal atresia had colonic replacement in the second and third year. One case of Hirschsprung's disease had the second stage in the second year and the rest were medical cases.

The triceps subcutaneous fold percentile distribution (Table 3) and not the subscapular one (Table 4) was significantly thinner in

Table 1 *Pertinent data concerning the number age range, mean age and sex in the different groups*

	Treated	Untreated	Sibling	Control	PDA ligated after 1st year
Number	77	35	24	75	10
Age range	0 81-10 53	0 87-11 55	1 10-9 13	0 92-11 01	2 40-9 49
Mean age	4 51	4 70	5 48	5 89	5 84
Males	44	18	11	37	6
Females	33	17	13	38	4

of children whose patent ductus arteriosus was ligated after the first year

Table 1 summarizes the pertinent data concerning different groups

Methods of measurement

The standing height was measured by a Harpenden Portable Stadiometer. Gentle upward traction was applied to the mastoid processes. The head was positioned so that the child was looking directly forward with the Frankfort plane (lower border of the orbit to the external auditory meatus) and the bi-auricular plane horizontal.

The supine length was measured by a Harpenden supine length table.

The weight was measured by an Avery weight chair which was accurate to the nearest 50 g. The child was weighed either nude or wearing his under pants only (no correction was made for these).

The head circumference was measured by a cloth

tape measure applied around the head touching the glabella anteriorly and the posterior occipital protuberance posteriorly. The measure was checked against stretching.

The chest circumference was measured by a cloth tape measure applied around the chest at the level of the xiphisternal joint midway between inspiration and expiration.

The triceps and the subscapular subcutaneous folds were measured by a Harpenden skin caliper according to Tanner & Whitehouse technique (24).

The skeletal age was assessed personally from an X-ray of the left hand and wrist using the Greulich & Pyle Atlas (10). Some of the X-rays were assessed by the local radiologists using the same atlas.

Standards used. Height and Weight. The percentile groups were obtained from Tanner's charts. The standard scores for height were calculated from the tables of Tanner et al. (25). The standard scores for weight were calculated from Scott's LCC (21) tables for

Table 2 *Total number and percentage of children below the 3rd percentile in height and weight in different groups and diagnostic sub groups*

Group	Total no	Height		Weight	
		n		n	
A Control	75			1	1.3
B Sibling	24			1	12.5
C Treated	77	19	24.0	21	28.0
Oesophageal atresia	16	6	37.5	8	37.5
Hirschsprung's disease	9	1	11.0	2	22.0
Intestinal obstruction	15	4	26.7	2	13.3
Duodenal atresia	7	2	28.6	1	14.3
Exomphalos	4	1	25.0		
PDA (ligated in 1st year)	7	1	14.5	2	28.6
Failure to thrive	14	3	21.4	7	50.0
Idiopathic hypercalcaemia	4	1	25.0	1	25.0
Nephrogenic diabetes insipidus	1				
D Untreated	35	17	48.6	19	54.2
Oesophageal atresia (1st stage)	2	1	50.0	1	50.0
Hirschsprung's disease + ileostomy	1	1	100.0		
Fallot's tetralogy	4	2	50.0	2	50.0
Acyanotic congenital heart disease	12	3	25.0	6	50.0
Mental deficiency	13	10	77.0	10	77.0
Fibrocystic disease of pancreas	3				
E PDA (ligated after 1st year)	10	1	10.0	2	20.0

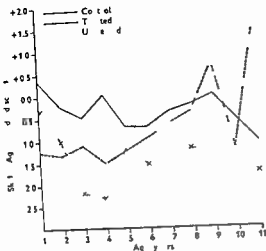


Fig 2 Age trends in mean skeletal age standard scores

ard scores) than males (-0.73 ± 1.04 standard scores) ($t = 12.10$, $p < 0.001$)

There was only slight differences in the post operative growth picture between cases of PDA ligated in and after the first year

Effect of birth weight and gestational age on subsequent height

The mean birth weight of a control sample consisting of 223 children born in the Jessop Hospital in 1961 was 3.202 ± 0.638 kg. The mean birth weight of the treated group was 2.834 ± 0.603 kg and it was significantly lower than that of the control birth weight ($p < 0.01$). The correlation between birth weights and heights was $r = +0.119$ (not significant). The correlation

between gestational ages and heights was $r = +0.039$. In the untreated group the mean birth weight was 2.926 ± 0.533 kg and was significantly lower than that of the control ($p < 0.01$). In the treated group 23% of the children had low birth weight (below 2500 g) and half of them were small for dates, while the incidence of low birth weight in the untreated group was 17% and all of them were small for dates.

Effect of mother's height on subsequent height of their children

Most of the mothers were measured personally

Treated group The mean of the mother's height (160 ± 7.0 cm) was not significantly lower than that of the control group (162.5 ± 5.1 cm). The correlation between the heights of mothers and heights of their children was $r = +0.344$ ($p < 0.01$). Mothers of children who were below the 3rd percentile had a slightly shorter mean height than mothers of children over the 3rd percentile.

Untreated group The mean of mother's height (160.8 ± 6.0 cm) was not significantly lower than that of the control group. The correlation between the heights of the mothers and the heights of their children was $r = +0.006$.

Effect of father's height on subsequent height of their children

Most of the data were obtained from the mothers

Treated group The mean of the father's

Table II Effect of age of onset of failure to thrive on physical growth in the treated group

Number between brackets denotes the number of children examined

Age of onset of failure to thrive	n	Height Standard scores		Weight Standard scores		Head circumference Standard scores	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Onset in 1st two months	57	-1.07	± 1.51	-0.93	± 1.07	-0.98 (54)	± 1.05
Onset in 3rd and 4th months	10	-1.02	± 1.25	-1.09	± 1.03	-1.06	± 0.85
Onset in 5th to 11th months	10	-1.15	± 0.98	-1.54	± 1.40	-1.00	± 0.90

Table 6 Means, standard deviations of control, treated untreated and sibling groups on follow up
The significance level compared with control group is marked by asterisks: *** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

Group	Height Standard scores		Weight Standard scores		Head circumference Standard scores		Skeletal age Standard scores	
	Mean (n)	S D	Mean (n)	S D	Mean (n)	S D	Mean (n)	S D
Control	+0.25 (75)	±0.79	+0.04 (73)	±0.91	+0.02 (75)	±0.98	-0.37 (70)	±1.18
Treated	-1.09*** (77)	±1.41	-1.03*** (76)	±1.05	-0.98*** (76)	±1.06	-0.98** (75)	±1.57
Untreated	-1.62*** (35)	±1.39	-1.86*** (35)	±0.93	-1.12*** (35)	±1.15	-1.67*** (35)	±1.39
Sibling	-0.14 (24)	±1.06	-0.42* (24)	±1.09	-0.55*** (24)	±1.04	-0.74 (23)	±1.68

treated and untreated groups when compared with that of the control group

The chest circumference percentile distribution (Table 5) was significantly smaller in the treated and untreated groups than that of the control group

B Using the standard scores

From Table 6, we observe that the means for weight, and head circumference of the sibling group were significantly lower than those of the control group. The means for height, weight, head circumference and skeletal age of the treated and untreated group were significantly lower than those of the control group. However, only the means for height and weight of the treated group were significantly lower than those of the siblings.

Since the sibling group consisted of siblings of the three main groups, height and weight of

the ill and healthy siblings of the treated group were compared. From Table 7 bigger differences were observed although they were not statistically significant due to the small size of the samples.

In the treated group, the mean height at each year (from 3 to 8 years) was significantly lower than the corresponding mean of the control group (Fig 1). The mean skeletal age of the treated group at each year was slightly lower than the corresponding mean of the control group (Fig 2).

The only significant sex difference in the treated group was that females had a smaller mean head circumference (-1.30 ± 1.01 stand

Table 7 Comparison between the ill and healthy sibling of the treated group

Groups	n	Height standard scores		Weight standard scores	
		Mean	S D	Mean	S D
Ill cases	12	-1.47	±2.28	-1.34	±1.56
Healthy siblings	12	-0.07	±1.00	-0.46	±0.93
Significance		$t = 1.86$	$p < 0.1$	$t = 1.50$	$p < 0.2$

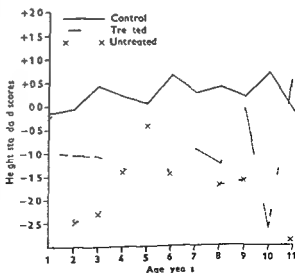


Fig 1 Age trends in mean height standard scores

Table 9 Effect of duration of failure to thrive on physical growth in the treated group

Duration of failure to thrive	n	Height standard scores		Weight standard scores		Head circumference standard scores	
		Mean	S D	Mean	S D	Mean	S D
Duration <4 months	12	-0.76	±1.53	-0.71	±1.01	-0.98	±1.23
Duration >4-8 months	10	-1.70	±1.34	-0.89	±1.41	-0.57	±0.98
Duration >8 months	18	-1.47	±1.34	-1.41	±0.94	-1.49	±0.86

tion increased in proportion to the duration of failure to thrive and a significant difference was found between the mean head circumference of the longest and moderate duration subgroups ($p < 0.001$). The incidence of low birth weight in the two shorter periods combined was 27% compared to 22% in the longest duration group.

Effect of age of correction of failure to thrive on subsequent physical growth in the treated group

Age of correction means the age at which the weight rose above the 3rd percentile in weight in the first year. From Table 10 we observe that there was minimal difference in the subsequent physical growth between early and late correction. However if the child's failure to thrive was not corrected during the first year there was more significant retardation in the three parameters tested ($p < 0.001$) than if the child's failure to thrive was corrected at any time in the 1st year.

The relation of age of correction of failure to thrive to the other variables is shown in Table 11. We observe that there was a high incidence of prolonged periods of failure to

thrive in the uncorrected group. Incidence of early onset of failure to thrive was similar in corrected and uncorrected groups. The older mean age of the uncorrected group might curtail their chance of catch up.

Effect of number of percentile channels lost during failure to thrive on subsequent physical growth in the treated group

Children with low birth weight were excluded. The number of percentile channels lost is an indication of failure to thrive. However losing 4-5 channels caused less retardation in the parameters tested than losing 1-2 channels.

DISCUSSION

It is difficult to exclude the effect of illness from that of genetic endowment. The ideal control group would consist of children of this group who were also below the 3rd percentile in weight in their first year but this would be practically impossible to collect.

The significantly retarded growth of the surgically treated cases did not conform to the within normal weight reported by Eckstein (5). The non significant differences between

Table 10 Effect of age of correction of failure to thrive on physical growth in the treated group

Age of correction of failure to thrive	n	Height standard scores		Weight standard scores		Head circumference standard scores	
		Mean	S D	Mean	S D	Mean	S D
<4th month	12	-0.76	±1.54	-0.71	±1.01	-0.93	±1.25
4th-1st month	7	-0.53	±1.49	-0.22	±0.60	-0.13	±0.83
Corrected in 1st year	19	-0.61	±1.48	-0.53	±0.89	-0.63	±1.15
Not corrected in 1st year	21	-1.72	±1.06	-1.65	±0.95	-1.54	±0.73

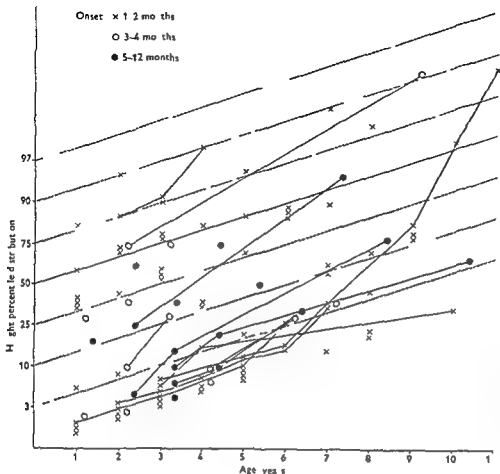


Fig 3 Effect of age of onset of failure to thrive on subsequent height percentiles in relation to chronological age. Lines joining the marks concern children at successive follow ups

height (171.5 ± 8.9 cm) was significantly lower than that of the control group (174.8 ± 8.0 cm) ($p < 0.01$). The correlation between the heights of the fathers and the heights of their children was $r = 0.300$ ($p < 0.02$). The mean of father's height of children below the 3rd percentile was similar to that of fathers of children above the 3rd percentile.

Untreated group: The mean of fathers height (171.3 ± 7.5 cm) was significantly lower than that of the control group ($p < 0.05$). The correlation between the heights of fathers and the heights of their children was $r = 0.799$.

Effect of age of onset of failure to thrive on subsequent physical growth in the treated group
Age of onset means the time at which the child's weight fell below the 3rd percentile in the first year. We observe from Table 8 that the very early onset of failure to thrive did not lead on the average to more retarded growth than later onset. To ensure that the hetero-

geneity of the diagnoses did not affect the results, means of height standard scores of cases of idiopathic failure to thrive with early (5 cases) and late (9 cases) onsets were calculated. Those with late onset were on the average slightly shorter than those with early onset. From Fig 3, we observe that with different times of onset there was no significant difference in the height percentiles at the final follow up at different ages. With onset in the first two months 14 cases were on or above the 50th percentile (24.6%) while 15 cases were below the 3rd percentile (26%) with a later onset 2 cases were on or above the 50th percentile (10%), while 4 cases were below the 3rd percentile (20%).

Effect of duration of failure to thrive on subsequent physical growth in the treated group

The duration here means the time spent below the 3rd percentile in weight in the first year. From Table 9 we observe that growth retarded

In the present treated group 19 cases were below the 3rd percentile

Since illness associated with failure to thrive especially when prolonged and at any time in the first year caused significant growth retardation every effort should be made to tide the child over that period to hasten his recovery and to shorten this period. In the surgically treated children this requires particular attention to early and late post-operative nutrition and adequate fluid and electrolyte balance

SUMMARY

This was a follow up study of physical growth of children who have had failure to thrive. It comprised 77 children in whom the cause was corrected and 35 children with persistent organic cause together with 10 children in whom the patent ductus arteriosus was ligated after the first year. The treated group consisted of surgical and medical cases. Their pattern of growth was compared with that of a control group composed of 75 children from the fracture clinic and 24 siblings all from the same age range and nearly the same mean age. Seven parameters of growth were tested. Significant retardation in six parameters was noted in both the treated group (in which the cause of failure to thrive was removed) and the untreated group (in which the treatment of the cause was impossible). In the treated group the mean skeletal age was less retarded than the mean height while the mean head circumference of females was significantly more retarded than that of males. There was only slight difference between early and late ligation of patent ductus arteriosus in subsequent physical growth. Neither the very early onset of failure nor its severity caused a severe retardation within the treated group. Prolonged duration of failure to thrive or failure to correct the cause in the first year led to significant subsequent growth retardation within the treated group. Parental height and birth weight played a less well defined role while socio-economic environment played a minor role in causing growth retardation

in the treated group. The first year can only be regarded as a relatively critical period concerning subsequent physical growth due to difficulty in separating the effect of illness from that of genetic endowment. Growth retardation may represent another congenital abnormality independent of the original anomaly which was corrected. The importance of reduced cell number was discussed.

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Table 11 *Comparison between corrected and uncorrected cases of failure to thrive in relation to other variables*

	Corrected in 1st year	Not corrected in 1st year
Number	19	21
Mean age	4 years	6 years
Cases with low birth weight (below 2 500 g)	5	5
Cases with early onset of failure to thrive	13	14
Cases with duration of failure to thrive more than 8 months	1	17
Medical cases	6	9
Surgical cases	13	12

early and late ligation of PDA agree with other reports (17-26)

The non significant retardation of skeletal age in comparison with the significantly shorter height was also reported (12) in children who had minor illnesses, although they were only able to follow their cases up to 4 years of age. This suggests that these children might end up as short adults unless the catch up in height exceeds that of skeletal age to overcome the early closure of the epiphysis. Prader et al (20) stated that the catch up in height is stronger than the catch up in skeletal age in these young children.

The significantly smaller mean head circumference of the females than that of the males in the treated group is in contrast with Tanner's (23) statement that females stand illness better than males.

The very low or even negative correlation of parental heights with the heights of children of the untreated group suggests that such genetic effect was inhibited by the persistence of the organic cause.

The significantly retarded growth of the sibling group suggests that its children came from a lower socio-economic environment than that of the control group. The significantly retarded growth of the treated and untreated groups compared to that of the sibling group suggests that such lower socio-economic en-

vironment was only partially responsible for their growth retardation.

CONCLUSION

Neither the very early onset of illness associated with failure to thrive nor its severity caused a greater degree of growth retardation within the treated group. Illness associated with prolonged duration of failure to thrive was the most major factor in producing significant growth retardation while the parental height, birth weight and socio-economic environment played a less well defined role. In spite of the fact that onset of failure to thrive at any time in the first year caused significant growth retardation compared to the growth of the control group, the first year can only be regarded as a relatively critical period since we cannot separate the effect of illness from that of genetic endowment.

Children with congenital heart disease fail to grow after the corrective cardiac surgery. These children have reduced cell number when this parameter was equated with the chronological age or height (4). Cell multiplication takes time, and too much time has been lost in these children—time when growth by multiplication was important.

Since congenital anomalies, which were corrected by surgery were the principal cause of failure to thrive in the treated group, it may be that the smaller size of these children was another congenital anomaly not dependent on the original anomaly.

The mean maximum serum growth hormone response in cases of dwarfism other than that caused by hypopituitarism was not significantly different from that response observed in a control group (15) while raised serum growth hormone levels were reported in cases of Kwahtorkor (18). However, serum growth hormone deficiency was reported in cases of dwarfism associated with emotional deprivation (19). The diagnosis of hypopituitarism is considered if the height was much below the 3rd percentile and lagged progressively below it (13).

In the present treated group 19 cases were below the 3rd percentile

Since illness associated with failure to thrive especially when prolonged and at any time in the first year caused significant growth retardation, every effort should be made to tide the child over that period to hasten his recovery and to shorten this period. In the surgically treated children this requires particular attention to early and late post operative nutrition and adequate fluid and electrolyte balance

SUMMARY

This was a follow up study of physical growth of children who have had failure to thrive. It comprised 77 children in whom the cause was corrected and 35 children with persistent organic cause together with 10 children in whom the patent ductus arteriosus was ligated after the first year. The treated group consisted of surgical and medical cases. Their pattern of growth was compared with that of a control group composed of 75 children from the fracture clinic and 24 siblings all from the same age range and nearly the same mean age. Seven parameters of growth were tested. Significant retardation in six parameters was noted in both the treated group (in which the cause of failure to thrive was removed) and the untreated group (in which the treatment of the cause was impossible). In the treated group the mean skeletal age was less retarded than the mean height while the mean head circumference of females was significantly more retarded than that of males. There was only slight difference between early and late ligation of patent ductus arteriosus in subsequent physical growth. Neither the very early onset of failure nor its severity caused a severe retardation within the treated group. Prolonged duration of failure to thrive or failure to correct the cause in the first year led to significant subsequent growth retardation within the treated group. Parental height and birth weight played a less well defined role while socio-economic environment played a minor role in causing growth retardation

in the treated group. The first year can only be regarded as a relatively critical period concerning subsequent physical growth due to difficulty in separating the effect of illness from that of genetic endowment. Growth retardation may represent another congenital abnormality independent of the original anomaly which was corrected. The importance of reduced cell number was discussed.

ACKNOWLEDGEMENTS

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INTENSIVE CARE OF SMALL PREMATURE INFANTS

II Postmortem Findings

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We have described previously the clinical features in 49 premature infants weighing less than 1250 g of whom 32 were treated with intermittent positive pressure respiration (IPPR) (31). This report concerns the clinical pathological correlation in the 27 infants who died such a specific study has not been reported before.

MATERIAL AND METHODS

Detailed postmortem examinations of 27 premature infants weighing less than 1250 g who had been treated with IPPR were done between 16 and 38 hours after death the clinical details of these cases have been described elsewhere (31). Specifically tissue blocks from the hilus to the pleural surface were taken from every pulmonary lobe. All tissue was fixed in neutral formalin and processed by standard paraffin techniques. Hematoxylin and eosin or van Gieson stain were used. Bacteriological specimens were taken from the posterior lobes of each lung. The following microscopic pulmonary abnormalities were noted: the extent thickness and stage of resolution of hyaline membrane and the presence of haemorrhage inflammatory cell infiltration and fibrous material in various parts of the lung. It was sometimes difficult to distinguish hyaline membranes from fibrous material but the basophilic staining of the latter and its involvement of bronchi were considered important points. The presence of focal parenchymal necroses and intra and interalveolar fibrosis was also recorded. These changes were graded as follows: The hyaline membranes and haemorrhages were graded on the approximate extent of histological involvement as + (<10%) to +++ (>50%). The

thickness of the membranes and the extent of their resolution as well as the density of the inflammatory cell infiltrate fibrosis and focal necroses were graded from slight (+) to severe (+++).

RESULTS

The clinical definitions symptomatology the diagnoses and the chest roentgenograms have been given previously (31).

Response to treatment

The material was divided into three categories: severe respiratory distress (RD), moderate RD and slight or no RD (31).

The diagnoses duration of IPPR and survival times are shown in Table 1. Of the 11 infants with severe RD 5 required immediate postnatal intubation and assisted ventilation and the other 6 required this within 1-14 hours (mean 6 hours). Ten died within 72 hours with little or no response to treatment as measured by arterial pO₂ and the clinical state. One died after 3 months having developed the clinical and roentgenological signs of bronchopulmonary dysplasia (BPD) (18).

A much better response to IPPR was found in those with moderate RD. IPPR treatment was needed less urgently (within 3-125 hours mean 44 hours) and the survival was longer. In the last category IPPR was required even later (6-96 hours mean 57 hours) in 7 cases as a result of sudden unexpected apnoea. The

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Table 1 *Clinical, radiological and autopsy findings, duration of IPPR and age at death in the 3 categories of RD*

RDS = respiratory distress syndrome HM = hyaline membranes PH = pulmonary hemorrhage IVH = intraventricular brain hemorrhage INF = infection BPD = bronchopulmonary dysplasia

Case no	Diagnosis		Chest X ray	Duration of IPPR (hours)	Age at death
	Clinical	Autopsy			
<i>Severe RD</i>					
23	RDS PH	HM (PH)	II	11	3 days
24	RDS	HM	II	17	17 days
25	RDS PH	HM	III	14	14 days
26	INF IVH	INF (IVH)	—	15	15 days
27	IVH	IVH	I	23	23 days
28	RDS	HM	III	7	13 days
29	RDS	HM (IVH)	III	22	1 day 2 hours
30	RDS	HM	III	24	1 day 4 hours
31	INF	INF	II	35	1 day 17 hours
32	RDS BPD	HM BPD	III	648	3 months
33	IVH PH	IVH (PH)	I	64	2 days 17 hours
<i>Moderate RD</i>					
34	RDS	HM (PH)	—	1	16 days
35	IVH pneumoth	IVH (pneumothorax)	I	240	15 days
36	IVH	INF (IVH PH)	—	2	1 day 1 hour
37	INF RDS	INF (PH)	II	192	9 days
38	RDS	IVH	I	34	1 day 14 hours
39	RDS PH	HM	II	98	4 days
40	RDS IVH	HM (IVH)	I	23	5 days
<i>Slight or no RD</i>					
41	Apnea pneumoth	HM (pneumothorax)	II	236	18 days
42	IVH pneumoth	HM (IVH pneumothorax)	II	168	11 days
43	INF RDS pneumoth	INF (IVH PH)	I	31	1 day 13 hours
44	Apnea Inf sec	INF	I	609	26 days
45	INF IVH	INF (IVH)	I	72	7 days
46	INF	INF	I	130	8 days
47	Apnea Inf sec	INF	I	264	45 days
48	INF RDS	INF (IVH)	I	6	21 hours
49	IVH	IVH	I	1	1 day 5 hours

initial response to treatment was good, but was followed by gradual deterioration. Despite survival of up to 45 days after prolonged IPPR, no evidence of BPD was found.

Chest roentgenograms

The chest X-rays in 9 cases of the *severe RD* group showed grade II or III diffuse reticulo-granularity and air bronchograms (31) characteristic of severe RDS. Two cases with grade I changes had brain haemorrhage, but no signs of pulmonary disease. In the category of *moderate RD* two of the five chest X-rays obtained showed grade II changes, these 2 patients were diagnosed as clinically RDS. Six of the eight

X-rays in the category of *slight or no RD* did not show any reticulo-granularity. In the 4 cases with infection, infiltrations indicating pulmonary infection were seen only in the later phase.

Autopsy findings

Pulmonary. Table 2 shows the material divided into four groups on the basis of autopsy data.

I Hyaline membrane group (HM) (Fig 1) Twelve cases were included in this group. The extent, thickness and degree of resolution of the membranes was variable, but otherwise lung morphology showed considerable similarities. Some inflammatory cells, both neutrophils and

Table 2 Bacteriologic and microscopic findings in the 4 autopsy categories (see text)

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Case no.	Hyaline membranes			Inflammatory cells			Fibrous material			Hemorrhages			Bacterial findings
	Distribution	Thickness	Reabsorption	Inflammatory cells		Peri bronchial	Fibrous material		Hemorrhages				
				Intra alveolar	Inter alveolar		Intra bronchial	Intra alveolar & bronchial	Inter peribronch	Focal necr			
<i>Hyaline membranes</i>													
23									+	+	-	-	-
24									-	-	-	-	-
25									-	-	-	-	-
26									-	-	-	-	-
27									-	-	-	-	-
28									-	-	-	-	-
29									-	-	-	-	-
30									-	-	-	-	-
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35									-	-	-	-	-
36									-	-	-	-	-
37									-	-	-	-	-
38									-	-	-	-	-
39									-	-	-	-	-
40									-	-	-	-	-
41									-	-	-	-	-
42									-	-	-	-	-
<i>Infection</i>													
43									-	-	-	-	-
44									-	-	-	-	-
45									-	-	-	-	-
46									-	-	-	-	-
47									-	-	-	-	-
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100									-	-	-	-	-

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Fig 3 Case 45 the alveoli and bronchiolar lumina are filled with fibrinous material (grade +++). Inflammatory cells are seen in the alveoli, the interalveolar septum and in the peribronchial tissue. HE. 170 magnification.

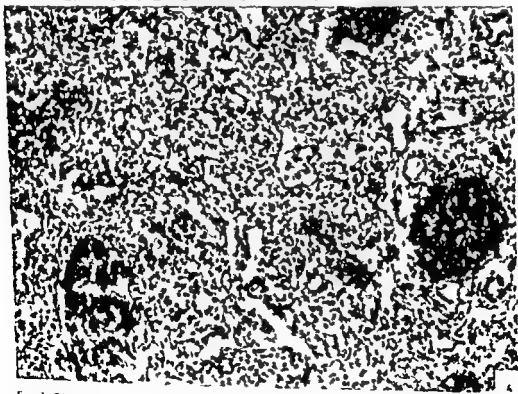


Fig 4 Case 4 pneumonia with numerous focal necroses (+++). HE. 170 magnification.

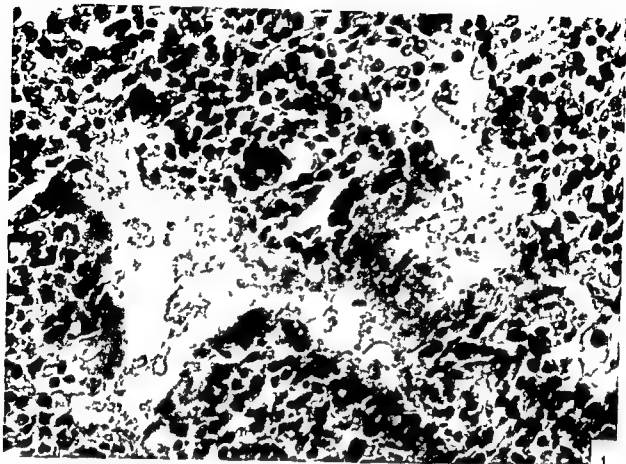


Fig 1 Case 34 an alveolus lined with hyaline membranes thickness ++ and resolution ++ Hema toxylin and eosin (HE) $\times 450$ magnification

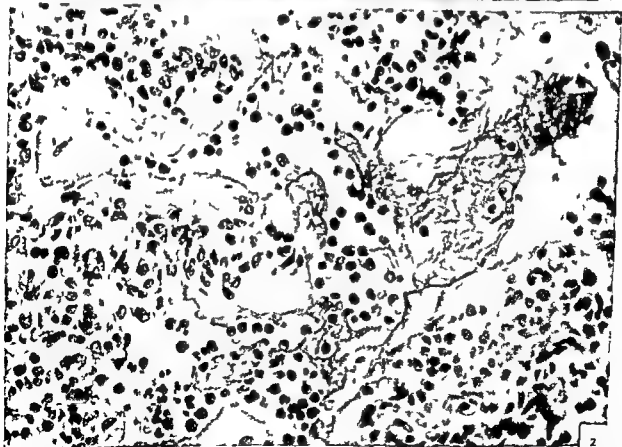


Fig 2 Case 42 an alveolus lined with hyaline membranes thickness ++ and resolution +++ In addition fibrinous material and inflammatory cells are in the alveolus HE $\times 450$ magnification

lymphocytes were seen in 4 of the 12 cases. The presence of intraalveolar inflammatory cells correlated with the extent of membrane resolution (Fig. 2). Significant inter alveolar or peribronchial inflammation was not seen. Case 32 showed pronounced intraalveolar inflammation and fibrosis as well as interalveolar septal fibrosis (Fig. 6). This case was clinically and radiologically diagnosed as suffering from bronchopulmonary dysplasia (18).

II Inflammation (INF) A histologic diagnosis of pneumonia was made in 10 cases (Fig. 3). In 6 of these there were small focal necroses in the lung parenchyma (Fig. 4). Neutrophilic and lymphocytic inflammatory cells were found in the interalveolar and peribronchial tissue as well as in the air spaces where a large amount of fibrinous material was also found. Aspirated amniotic material was never found. Bacteriologic investigation revealed gram negative bacilli *E. coli*, *Klebsiella* and *Pseudomonas* in 5 and *Haemophilus influenzae* in 1 of the cases.

In 6 of the 10 cases early rupture of the fetal membranes associated with foul smelling amniotic fluid at birth suggested intrauterine infection. Death before the age of 48 hours in 3 of these (nos. 26, 31, 48) was further evidence suggesting intrauterine infection. In cases 36 and 43 copious amounts of fibrinous material were present in the airspaces but no focal

Table 4 Clinicopathological correlation in 27 cases of RDS

Definitive diagnosis	Correct clinical diagnosis	Wrong clinical diagnosis	Missed clinical diagnosis	Total definitive diagnosis
RDS/HMD	10	4	2	12
Pneumonia	9	—	1	10
Massive IVH	9	—	4	13
Massive PH	2	2	4	6

necroses were seen and no organisms were found in bacteriologic investigation. Clinically these cases had been diagnosed as having RDS but no hyaline membranes could be seen on histologic examination. Thus despite the clinical history the histologic picture and the early deaths at 25 and 37 hours of age respectively suggested intrauterine or perinatally acquired infection.

In the last 2 cases (nos. 44 and 47) inflammatory cells were seen only within the air spaces and no focal necroses were observed. Clinically these infants suffered severe apnoeic attacks when 15 and 55 hours old inflammatory symptoms only appearing after prolonged IPPR. The age at death in these cases was 45 and 26 days which was considerably greater than in the other eight infectious cases.

III Pulmonary hemorrhage (PH) Only 1 case (no. 33) showed signs of a massive pulmonary hemorrhage as a single lesion of the lungs. In this case the hemorrhagic area involved 20% of the total area of the sections. In the HMD cases pulmonary congestion was accompanied by microscopic hemorrhages of varying degree in 6 of the 12 cases (Fig. 5).

In the infected lungs microscopic areas of hemorrhage anatomically contiguous were seen in 5 of the 10 cases, 3 of whom also had focal necroses. An additional case showed generalized intrapulmonary hemorrhage.

IV No pulmonary lesions In 4 cases (nos. 27, 35, 38 and 49) with massive intraventricular brain hemorrhage no pathologic features could be demonstrated in the lungs.

Intraventricular brain hemorrhage A massive intraventricular brain hemorrhage (IVH)

Table 3 Major pulmonary and associated post mortem findings in RDS (27 cases)

Pulmonary finding	Associated finding	No. of cases
Hyaline membranes (11 cases)	Massive IVH	3
	Massive PH	2
	None	7
Pneumonia (10 cases)	Massive IVH	3
	Massive IVH PH	2
	Massive PH	1
	None	4
Pulmonary hemorrhage	Massive IVH	1
No pulmonary abnormality	Massive IVH	4

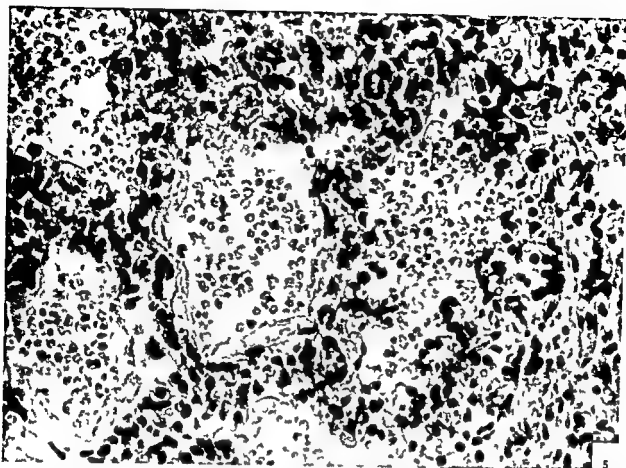


Fig 5 Case 23 intra alveolar hemorrhage combined with hyaline membranes HE $\times 450$ magnification

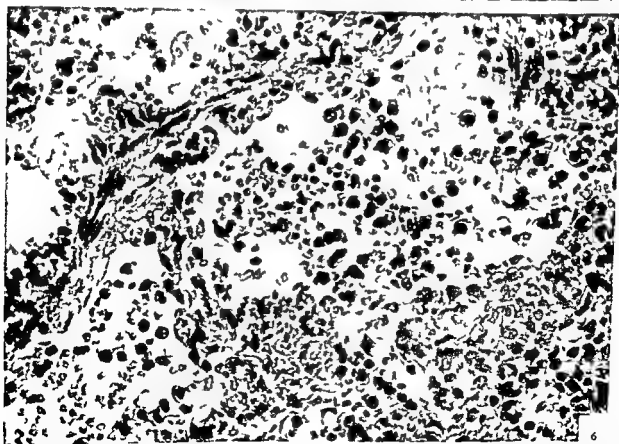


Fig 6 Case 32 bronchopulmonary dysplasia with fibrosis of the interalveolar septa HE $\times 450$ magnification

were found in most cases of hyaline membranes or infection

Massive brain hemorrhage Of the 13 cases with this finding in only 4 were there no pulmonary abnormalities. In these 4 cases the brain hemorrhage was considered the cause of respiratory failure. In the remaining 9 cases the pulmonary abnormalities were considered to be the primary ones and accordingly they were classified on these findings.

Pulmonary effect of IPPR and oxygen treatment There are reports that IPPR treatment may be harmful. IPPR has been suggested to cause local lung damage (24, 26, 29) as well as circulatory changes which may predispose to IVH (1, 7, 25, 28). High concentrations of oxygen may also produce acute pulmonary damage (14, 18, 22, 23, 24, 25, 29, 30) and in severe RDS Northway et al. have suggested that it may result in later "bronchopulmonary dysplasia" (18). Our results are compatible with Northway's view in that the sole severe case of RDS to survive 3 months showed these changes while 1 in severe or infected cases did not. On the other hand we did not see any pulmonary lesions which have not been described in infants not treated with IPPR. An unusual finding was the high incidence of IVH (5 of 8) in those infants with intra uterine infection however as this was not matched by a similarly high incidence in the RDS group IPPR treatment is unlikely to have been an important cause of it.

SUMMARY

Twenty seven deaths in premature infants weighing less than 1250 g who were treated with intermittent positive pressure respiration are reported. The major pulmonary abnormalities were hyaline membrane formation, infection and hemorrhage occurring alone or in combination with intraventricular hemorrhage. Hyaline membranes were associated with severe clinical features of respiratory distress whereas infection was not. Prolonged treatment resulted in bronchopulmonary dysplasia in only 1 case

where severe clinical respiratory distress was associated with the finding of hyaline membranes at post mortem.

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was found in 13 cases. As shown in Table 3, this was the only major finding in 4 cases. In case 33, an isolated massive pulmonary hemorrhage was found accompanying a massive IVH. Five of the 10 cases of infection also had a massive IVH in contrast to only 3 of the 12 cases of RDS.

Correlation of clinical and autopsy findings

Table 4 shows the incorrect or missed clinical diagnoses. There seems to be a tendency to overdiagnose hyaline membranes and pulmonary hemorrhage (4 and 2 cases respectively) whereas all cases diagnosed as having IVH and infection were confirmed at autopsy. On the other hand, there were 4 cases of combined IVH and PH in which the diagnosis was not made before death.

There was a good correlation between the severity of clinical RDS and the distribution and thickness of the pulmonary hyaline membranes found at autopsy. Thus, cases allocated to the category of *severe RD* also had the most pronounced hyaline membranes (Table 1 and 2). The occurrence of massive IVH however was not correlated with the severity of the respiratory symptoms. There were 2 cases of infection in the categories of *severe RD* and *moderate RD* and 4 cases in the category of *light or no RD*.

DISCUSSION

The present study reports the pathological findings in infants treated with IPPR. The series could be divided into four categories on pathological criteria.

Pulmonary hyaline membranes. In this category hyaline membranes were practically the sole finding. Other authors (11, 13, 15, 33) have stressed associated pulmonary vascular congestion but this, as well as gross hemorrhage, was less frequent in our cases. Inflammatory cells were related to resolution of the membranes and could therefore represent a reaction to this process as has been suggested by others (13, 16, 21, 32, 33, 24). The negative

bacterial cultures in 11 of the 12 cases is evidence against an infective course for the lesion and furthermore, the cell distribution in this group and that with proven infection without membranes was different. Interstitial inflammation which Olding (10) considered essential to a diagnosis of bacterial infection, being rare in those with hyaline membranes alone.

Chest radiography is one of the best diagnostic aids in neonatal respiratory disorders (3, 10, 12, 20). The degree of reticulo granularity on chest roentgenograms correlated well with the autopsy occurrence of pulmonary hyaline membranes (4, 9). However, minor degrees of hyaline membranes were found at autopsy in the less severe cases which had not shown these radiographic features.

Pulmonary infection. In 10 cases the pulmonary lesions were considered to be due to infection. In 2 of these cases this may have resulted from prolonged IPPR. Both showed limited fibrinous material and cell infiltration in the airspaces. In both cases gram negative organisms were found. In the remaining 8 cases 6 had a history of early rupture of the fetal membranes including 3 with evidence of criminal induction of labor. In these cases, a characteristic finding seemed to be focal necroses in the lung parenchyma. This specific abnormality has been attributed to gram negative bacteria (27) and in 5 of the 6 cases with this lesion such organisms were isolated. All of them had much fibrinous material in the airspaces. These findings are quite different to the diffuse inflammatory process with evidence of aspiration and little fibrinous material in the airspaces considered characteristic of neonatal pneumonia (6, 17, 27).

Pulmonary hemorrhage. Extensive pulmonary hemorrhage unrelated to hyaline membranes or other pulmonary pathology is a common finding in newborn infants at autopsy (2). However as the sole lesion this was found in only 1 case in this series. In the other cases (Table 3) it accompanied pulmonary hyaline membranes (2 cases) or infection (3 cases). In addition, restricted areas of microscopic hemorrhage

were found in most cases of hyaline membranes or infection

Massive brain hemorrhage Of the 13 cases with this finding in only 4 were there no pulmonary abnormalities. In these 4 cases the brain hemorrhage was considered the cause of respiratory failure. In the remaining 9 cases the pulmonary abnormalities were considered to be the primary ones and accordingly they were classified on these findings.

Pulmonary effect of IPPR and oxygen treatment There are reports that IPPR treatment may be harmful. IPPR has been suggested to cause local lung damage (24, 26, 29) as well as circulatory changes which may predispose to IVH (1, 7, 25, 28). High concentrations of oxygen may also produce acute pulmonary damage (14, 18, 22, 23, 24, 25, 29, 30) and in severe RDS Northway et al. have suggested that it may result in later bronchopulmonary dysplasia (18). Our results are compatible with Northway's view in that the sole severe case of RDS to survive 3 months showed these changes while less severe or infected cases did not. On the other hand we did not see any pulmonary lesions which have not been described in infants not treated with IPPR. An unusual finding was the high incidence of IVH (5 of 8) in those infants with intra uterine infection however as this was not matched by a similarly high incidence in the RDS group IPPR treatment is unlikely to have been an important cause of it.

SUMMARY

Twenty seven deaths in premature infants weighing less than 1250 g who were treated with intermittent positive pressure respiration are reported. The major pulmonary abnormalities were hyaline membrane formation, infection and hemorrhage occurring alone or in combination with intraventricular hemorrhage. Hyaline membranes were associated with severe clinical features of respiratory distress whereas infection was not. Prolonged treatment resulted in bronchopulmonary dysplasia in only 1 case

where severe clinical respiratory distress was associated with the finding of hyaline membranes at post mortem.

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was found in 13 cases. As shown in Table 3, this was the only major finding in 4 cases. In case 33 an isolated massive pulmonary hemorrhage was found accompanying a massive IVH. Five of the 10 cases of infection also had a massive IVH in contrast to only 3 of the 12 cases of RDS.

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RECONSTRUCTION OF OESOPHAGUS WITH COLONIC TRANSPOSITION

G GRÖTTE L. OLSEN and A. REUTERSKJÖLD

From the Department of Paediatric Surgery, University Hospital, Uppsala, Sweden

The replacement of defective parts of the oesophagus has always been a major surgical problem. A large number of operative methods and modifications have been tried since Bircher (2) in 1894 performed the first oesophageal reconstruction using an antethoracic skin pedicle. Some few methods have gradually crystallized and these are discussed below.

Relatively few series of patients are reported in the Scandinavian literature where the whole or parts of the oesophagus have been replaced by colonic transplants. We therefore present and discuss below a series of 6 children operated on during the last 4 years at the University Hospital, Uppsala.

MATERIAL AND METHODS

Of the 6 patients 5 had oesophageal atresia. Three of them underwent acute operation with direct end-to-end anastomosis; however, sutural insufficiency necessitated ligation of the distal oesophagus and cervical oesophagostomy. The other 2 with atresia had more than 10 cm distance between the oesophageal ends to allow primary direct anastomosis. The 6th child finally underwent operation for severe left thoracic haemorrhages from oesophageal varices.

CASE REPORTS

Patient 1 M G. Oesophageal atresia with tracheal fistula. Operation with end-to-end direct anastomosis and closure of the fistula. On the 4th day postoperatively there was leakage through the anastomosis and the distal oesophagus was therefore closed and cervical oesophagostomy performed. Fed via gastrostomy

and reoperated at the age of 14 months with retrosternal transposition of colon. On the 10th day postoperatively a cervical fistula developed but this healed spontaneously. Subsequent course free of complications and the patient has now been followed up for 3 years and 8 months since the operation (see Table 1).

Patient 2 I B. Oesophageal atresia with fistula to trachea. Operation on the first day of life with end-to-end anastomosis and division of the fistula. After 3 days reoperation for leakage in the anastomosis with closure of the distal oesophagus and transposition of the proximal part to the neck. Gastrostomy. At the age of 13 months colonic transposition in the same way as in patient 1. Complication free course during the observation time of 3 years and 8 months (see Table 1).

Patient 3 J F. Oesophageal atresia with tracheal fistula. Acute operation with end-to-end anastomosis and closure of the fistula. On the 2nd day rupture of the thoracic wound necessitating resuture. On the 5th day there was sutural insufficiency at the anastomosis and the oesophagus was therefore closed and cervical oesophagostomy performed on the proximal part. At the age of 8 months colonic transposition was made as above. Endotracheal intubation for bronchopneumonia with respiratory insufficiency 3 days postoperatively. After a further 4 days a cervical fistula developed with considerable leakage. Tracheostomy had to be performed. After 2 1/2 months he was discharged from hospital with a fenestrated silver cannula. After a further 3 months during which time he was looked after at home the cannula was removed with a good result. Essentially free of complications during the subsequent observation period and it is now 3 years and 4 months since the operation (see Table 1).

Patient 4 T N. Oesophageal atresia with tracheal fistula (from the proximal segment). The distal segment was very short and direct anastomosis was impossible. The fistula was divided and the proximal part of the oesophagus was transposed to the neck. Gastrostomy was performed. Was then nursed at home

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esent symptoms

iffic swallowing solid food borborygmus
n chest at times Loose stools sometimes
mucous not ejectile
ccasional stomach pains Borborygmus (much
ess than initially) Catches cold rel easily
ough Loose stools frequent 2/day and
only slight subjective symptoms

iffic swallowing solid food if poorly chewed
-light infections (upper respiratory) borborygmus

earburn acid regurgitation retrosternal
burning pain

el abundant muc secretion in throat
Diff in learning to swallow

mal segment the transplant is sutured distally to the
anterior side of the stomach on the fornix near to the
lesser curvature Pyloropasty according to the Finney
technique is always performed as well as gastros-
tomy

RESULTS

Up to now the results in our series have been very encouraging. No deaths have occurred and the first five patients are living an essentially normal life and have only minor discomforts. The last patient has stricture tendency at the upper anastomosis but the observation time is too short to make a prognosis. Two of the patients complain of difficulties in swallowing solid and inadequately chewed food. Three of them are troubled by moderate borborygmus retrosternally while only one patient has had symptoms indicating reflux with heartburn etc (see Table 1). Finally 2 of the patients have loose stools but both have only relatively minor subjective symptoms.

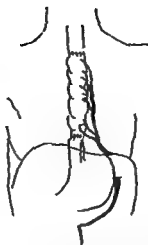


Fig 1 Technique according to Waterstone. Colon segment here placed dorsally within the left pleural cavity. Vascular pedicle coming up dorsally through the diaphragm. Lower colon anastomosis to oesophagus with preservation of cardia.

DISCUSSION

Since Bircher (2) made the first reconstruction of the oesophagus with an antethoracic skin pedicle in 1894 (published 1907) a large number of modifications have been attempted with the aim of replacing anomalous or destroyed parts of the oesophagus.

Kelling (5) for example performed the first colonic transposition in 1911.

In Sweden Lundblad (8) performed in 1920 an antethoracic colonic transposition in a 3



Fig 2 Retrosternal extra pleural technique. Lower colonic anastomosis to the ventricle.

Table 1 Summary of case reports

Pat	Primary disease	Age at op	Immediate postop complications	Postop obs time years	The values are for all the patients within normal limits (height weight)		X ray
M G 640716	Oesoph atresia with fist to trachea	14 mo	10th day cervical fistula (healed spontan)	3 8/12	100 cm 16 kg		Good passage
I B 640820	Oesoph atresia with fist to trachea	13 mo	0	3 8/12	100 cm 15.5 kg		Good passage
J E 640729	Oesoph atresia with fist to trachea	18 mo	3rd day intub for resp diff 7th day cerv fistula Tracheostomy with fenest silver cannula 5 1/2 mo	3 4/12	102 cm 15 kg		(Not done)
T N 650923	Oesoph atresia with fist to trachea	13 mo	5th day cervical fistula Healed spontaneously	2 7/12	93 cm 12.5 kg		Good passage
S W 570226	Banti's disease with oesoph varices	10 4/12 years	0	1 11/12	135 cm 25 kg		Moderate constriction but good passage
A A 680508	Oesophageal atresia No fistula to trachea	8 mo	Bilateral pneumothorax immediately postop 7th day cerv fistula healed spontaneously	4/12	70 cm 8.1 kg		Moderate constriction at upper anastomosis. Passage satisfactory

and had several attacks of upper respiratory infection and gradually also left sided exudative pleuritis which healed without complications. Operation with colonic transposition at the age of 13 months the same technique being used as in the above patients. On the 5th day a cervical fistula developed from the proximal anastomosis 3 1/2 months postoperatively there was a sudden onset of difficulty in swallowing with vomiting attacks but no pathological changes were observed in the transplant. Relatively rapid and spontaneous improvement and now 2 years and 7 months after the operation the patient is in a good general state of health (see Table 1).

Patient 5 S B. At the age of just under 2 years there was an onset of melena. After extensive investigations liver cirrhosis with portal hypertension was diagnosed. Partial gastrectomy was performed 1 1/2 years later local ligation of varices in the caudal region was performed. After one further year of severe haemorrhages a spleno renal shunt was carried out. Also after this operation there were repeated haemorrhages from large oesophageal varices. At the age of 9 1/2 years an anastomosis was made between the vena cava and the superior mesenteric vein. Despite this the haemorrhages recurred and the transfusions became increasingly difficult because of the child having pronounced hypersensitivity reactions. At 10 years and 4 months the lower part of the oesophagus and half of the stomach were resected and about

1 dm of transverse colon was transposed to the gaster thus produced. No further bleeding. One year and 1 month has now elapsed since the operation and the patient is in a good general condition (see Table 1).

Patient 6 A A. Born with oesophageal atresia with no fistula to the trachea and with a large distance between the oesophageal segments. Direct anastomosis technically impossible. Fed by gastrostomy. Attempts for several months at dilating the proximal pocket with a rubber bougie gave no results and finally colonic transposition was performed at the age of 8 months. Rather complicated postoperative course and artificial ventilation was necessary for 1 week. A cervical fistula occurred on the 7th day but healed spontaneously. The observation time is now only 8 months and it is therefore too early to make a prognosis.

Operative technique

We have used the retrosternal technique in a one stage procedure as follows.

The cecum and transverse colon are mobilized and a pedicle with the middle colic artery is utilized. The cecal pole which is usually too thick to allow a good cervical anastomosis is resected. The transplant which in 5 of the cases mainly comprised the ascending colon and in 1 case the transverse colon is then placed isoperistaltically through a retrosternal tunnel. After end to end anastomosis with the proxi-

Louhimo et al (7) have experienced difficulties for their children in gaining weight. They did not use pyloroplasty in any of six cases which may then be of importance in this connection.

Age. As regards a suitable age for the operation Gross & Firestone (3) consider that patient should be at least one year old. Both Waterston (15) and Rehbein et al (11) consider optimal age to be about 6 months for the reason that the thorax can then be easily reached through an incision at the level of the seventh thoracic vertebra and that the vascular term at that age is sufficiently well developed to give a good pedicle to be obtained. Othersen & Clatworthy (9) on the other hand prefer to wait until the age of about 18 months since the child can then be expected to sit relatively steadily and reflux after feeding thereby prevented. Linder & Hecker (6) also consider the best age to be during the second year of life.

Three of our patients were about 1 year of age at the time of operation. One patient was only 6 months old, another was 8 months and one just over 10 years (see Table 1). The most troublesome symptoms postoperatively occurred in the 6 month-old child. We consider that the most suitable age for operation is about 1 year. Theoretically it should be possible however to perform the operation earlier since at least metabolically an earlier operation should be well tolerated. Immunologically there is probably no difference of importance from about 6 months of age and upwards, neither as regards humoral nor cellular immunity especially concerning coliform organisms.

For patients with oesophageal atresia we consider that the operation should not be deferred much later than the age of 1 year because of the great inconveniences of feeding through a gastrostomy for a long period.

Iso- or antiperistaltically? Linder & Hecker (6) place their transplants both iso- and antiperistaltically and Gross & Firestone (3) do the same. Waterston (15) uses mainly isoperistaltic colon, however. Othersen & Clatworthy (9)

consider that it is of no significance how the intestine is placed in this respect. Of considerably greater importance is the blood supply to the intestine, the length of the transplant and its diameter etc.

We have placed the colon isoperistaltically in all cases and consider that this question most probably is of no importance for the results.

The few experimental investigations that have been made indicate that the isoperistaltic intestine functions somewhat more efficiently than the antiperistaltic. The peristalsis is very slow, however. Sherman & Waterston (14) have observed deep peristaltic waves of the colonic type in contrast studies. Other investigators e.g. Schafer (13) have also demonstrated oscillatory contracting like movements roentgenologically. Rehbein et al (11) have observed both these types of peristalsis.

In our opinion however peristalsis plays a very minor part in this connection and the most important factor is the force of gravity—which has also been pointed out by Othersen & Clatworthy (9).

Gastrostomy. We have performed gastrostomy in all cases and there seem to be no divided opinions on this question among other authors.

Stricture—bouginage? Rehbein et al (11) discuss the tendency of the transplant to become dilated postoperatively. They attribute this partly to the fact that the transplant was too long from the start, partly to a functional stenosis in the distal anastomosis due to the difference in calibre and finally to the effect of reflux (in operations by the retrosternal technique). He therefore dilates the distal anastomosis postoperatively by means of a continuous silk thread which is placed in position during the operation.

This stricture can often however be only a relative constriction as an expression of the intrathoracic dilation of the colon (due to the negative intrathoracic pressure) and the intra-abdominal constriction (due to positive intra-abdominal pressure). If roentgenography shows an apparent passage of food and the patient

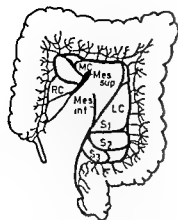


Fig 3 Showing right (RC) left (LC) and middle (MC) colic arteries and sigmoid arcs S1-3 (according to Griffiths 1936)

year-old child with a good result, Sandblom (12) made an attempt at intrathoracic colonic transposition in a 2 day old child in 1947 and Pettersson (10) performed the first successful intrathoracic colonic transposition in a newborn (1-day old) child in 1959

Most authors seem to agree that the colon has greater advantages as a replacement for the oesophagus than, for example the small intestine or mobilization of the stomach. At present in principle two methods are used, in which a part of the colon is interposed between the proximal oesophagus and distal oesophagus or stomach (Figs 1 and 2). The colon can either be placed retrosternally (as recommended by Gross & Firestone (3) among others) or it can be placed in the left pleural cavity behind the root of the lung (as performed by Waterston (15)). In this clinic the former method has been used.

Waterston (15) and Rehbein et al (11) use transverse colon isoperistaltically on a pedicle with the left colic artery. They make the distal anastomosis with the distal oesophagus and thus preserve the cardia.

Sherman Jr (14) on the other hand uses ascending colon isoperistaltically by the retrosternal technique.

Gross & Firestone (3) also favour the retrosternal technique, which is technically simpler, gives better lung function postoperatively and, most important of all, it keeps the pleural cavity

clean in the event of leakage. At present they prefer the descending colon, they consider that this has the advantage that a longer segment can be mobilized. We have had no technical problems, however, in getting the ascending colon, lying isoperistaltically on a pedicle with the middle colic artery to be of sufficient length.

Linder & Hecker (6) describe 5 cases where they used either ascending transverse or descending colon with the retrosternal technique (Fig 3).

In the first 5 cases we resected the caecal pole. In the last patient however, we retained the caecum and divided the colon just above Bauhin's valve. The reason for this was that two of the patients had been found on follow up to be having loose stools. One possible explanation for this may be that Bauhin's valve has an inhibitory effect on the propulsion through the intestines and that caecal resection would thus result in too rapid propulsion with consequent diarrhoea. However according to recent investigations by Johansson & Nylander (4) Bauhin's valve has no such action its function being to prevent the contents of the colon from passing in a retrograde direction. The diarrhoea might instead be explained by an infection ascending to the small intestine. We therefore intend to retain the caecal pole in future cases.

We consider that another detail of great importance is that the transplant should be made as straight as possible i.e. without kinks, especially in the distal part so as to prevent obstruction. This has also been pointed out by Rehbein et al (11) who have tried to make the transplant so short that it lies 'like a rubber band under tension'. Gross & Firestone (3) are also of a similar opinion.

In agreement with most authors we also perform pyloroplasty in order to facilitate evacuation of the stomach postoperatively. This may be of great importance in preventing reflux of acid gastric juice up into the transposed colon which may result in ulceration, bleeding and even perforation.

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has no discomfort, it is therefore our firm opinion that bouginage should be avoided. Gross & Firestone (3) also advise against bouginage and instead suggest reoperation with revision of the anastomosis in the event of postoperative obstruction.

In none of our 6 patients was bouginage of the distal anastomosis necessary. Patient 6, however, has a tendency to stenosis in the proximal anastomosis, which requires bouginage postoperatively.

Reflux. One of the main differences between the retrosternal and the intrathoracic technique is that in the latter case the distal anastomosis is made between the colon and oesophagus immediately above the cardia, the function of which is then retained. Waterston (15) aimed at preserving the function of the cardia in his series which, however, meant a higher operative risk. However, in a large number of his patients he does not seem to have obtained satisfactory cardiac function (personal communication).

One difficulty with Waterston's (15) technique is to produce a hole in the diaphragm large enough so as not to have any pressure effect on the pedicle, with a risk of necrosis of the transplant, but not so large that herniation can occur.

In spite of the relatively large number of operations that have now been performed by the retrosternal technique strikingly few cases have been reported with peptic changes in the transplant due to reflux (Battersby 1).

Further, with the retrosternal technique we have had no major problems of the reflux oesophagitis type. Only one of the patients (S W) has had symptoms of this, and they have been very mild. We have observed no ulceration in the colonic transplant on postoperative roentgenography.

Cervical fistulae. Cervical fistulae are a common complication according to most authors. Gross & Firestone (3) reported 6 fistulae all of which healed, in their series of 47 patients and Othersen & Clatworthy (9) reported 4 fistulae in 11 patients.

Of our 6 patients, 4 had cervical fistulae on the 5th to 10th day, all of which closed spontaneously, however, and have since caused no trouble to the patients.

Operative risk. The average mortality in some of the larger series with colonic transposition, performed for benign diseases in the oesophagus is around 10%.

The series reported here is small and there is no mortality.

SUMMARY

With the increased experience which has been gained during recent years, transposition of the colon can be regarded as a valuable method when for various reasons it is desirable to replace the whole or parts of the oesophagus.

A series of 6 patients operated on by the retrosternal technique with good results is reported.

On the basis of this series the advantages and disadvantages of this method are discussed and compared with other types of replacement of the oesophagus by colonic transposition.

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Table 1 Clinical data of our patients suffering from ataxia telangiectasia

Pat no	Age in years	Age at the onset of cerebellar ataxia (years)	Age at the onset of ocular telangiectasia	Skin signs	Infections	Other members of family with ataxia telangiectasia
1 Sz Zs	14	4	Newborn period	Cutaneous telangiectasia vitiligo lentigo	Repeated pneumonia bronchiectasia chronic otitis vulvitis	—
2 L D	10	Infancy	Newborn period	Cutaneous telangiectasia café au lait spots follicular hyperkeratose lentigo	0	—
3 J P	10	1½	5 years	Follicular hyperkeratose	0	Sister
4 L H	11	6	5 years	Cutaneous telangiectasia café au lait spots follicular hyperkeratose lentigo	Repeated pneumonia chronic otitis pleuritis	—
5 S Sz	9	3	1½ years	Cutaneous telangiectasia	Frequent common cold pneumonia two times otitis	—
6 C Sz	13	9	1½ yr	Café au lait spots follicular hyperkeratose lentigo	Repeated pneumonias chronic otitis bronchiectasia urogenital infection	Sister
7 F T	10	3	4 years	Cutaneous telangiectasia lentigo	Chronic otitis chronic mastoiditis sinusitis maxillaris	Sister

for provocation (1:100 in negative cases) Aisenberg's (1) or Young's (45) technique was employed for the measurement of DNCB skin reactions. Intra-dermal tuberculin tests were performed with an 1:10 dilution of tuberculin PPD and in cases of positive reaction another test with an 1:1000 dilution followed. The reactions were read after 72 hours.

The clinical data of our patients are shown in Table 1.

RESULTS

The rates of blast transformation and the mitotic indices (number of mitoses per one thousand cells) are to be found in Table 2. The values listed represent the results obtained from cultures containing AB Rh positive serum. In cubation with the patient's own serum gave values for both blast transformation and mitotic index slightly lower but not significantly different from the results with AB Rh positive serum. Generally blast transformation and

mitotic index were found to be depressed in ataxic patients but reached statistical significance only in respect to the number of mitoses. However in ca. 3 neither the blast transformation rate nor the mitotic index was different from that of the controls. This can be seen in the partial overlapping of the values too. In one of our cases (Zs Sz) four examinations were performed in 2 years where the degree of the depression of blast transformation proved to vary in the course of the disease (Table 3). No correlation was found between the plasma IgA levels or the results of the delayed type skin reactions and the decreases in blast transformation. No correlation appeared between these values and the occurrence of chronic recurrent infections either (Table 2).

The incorporation of ^3H thymidine also

LYMPHOBLASTIC TRANSFORMATION, CHROMOSOME PATTERN AND DELAYED TYPE SKIN REACTION IN ATAXIA TELANGIECTASIA

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The syndrome of conjunctival vasodilatation and cerebellar ataxia was first described in 1941 by Louis Bar (25) and was called ataxia telangiectasia. The frequent occurrence of sino-pulmonary infections in this condition was pointed out by Boder & Sedgwick (3), and Centerwall & Miller (4) independently in 1958. The diminution or lack of serum IgA level was first demonstrated on a large material by Thieffry et al in 1961 (42). In 1963 Peterson et al (29) suggested that the immunological deficiencies in patients with ataxia telangiectasia may be related to abnormal thymic function. This suggestion has been subsequently confirmed.

A decreased blastogenic transformation of lymphoid cells was observed by Leikin et al (23) and has been confirmed by numerous authors (17, 20, 21, 24, 27, 28). In one of our patients, however, we found lymphocytic blastogenic transformation to be almost normal (37), we therefore repeated this examination on six further cases.

MATERIAL AND METHODS

Blastic transformation was studied in peripheral leucocyte cultures by the slightly modified method of Moorhead (26, 36). The total volume of each culture amounted to 10 ml/742 ml TC 199 (Difco) culture medium + 2.0 ml human plasma + 0.5 ml cell suspension + 0.08 ml phytohaemagglutinin + 10 mg

streptomycin + 10 000 E penicillin. Incubation was carried out both with AB Rh positive plasma and with the patient's own plasma. After 72 hours 0.08 ml of a 0.04% colchicine solution was added to each culture. After 2 hours harvesting hypotonication (0.5 ml TC 199 + 2.0 ml distilled water fixation (with acetic acid + methanol) and flaming and drying were then performed. The morphological features distinguishing lymphoblasts was a large loosely structured nucleus with occasional nucleoli; nontransformed cells were characterized by intensely stained, nearly black amorphous bodies (15). Transitional forms were classified as blasts. In every culture 1 000 cells were counted by each of three examiners.

Chromosomal studies were carried out only on well defined metaphases. Since in ataxia individual the number of mitoses is reduced, chromosomal evaluation could be performed in only 4 cases in 11 cells together.

The incorporation of ^3H thymidine into nuclear DNA was studied in 3 cases. To the nutrient medium (2.5 ml) consisting of TC 199 (Difco) and 20% AB Rh positive human plasma, 0.02 ml of phytohaemagglutinin P was added. Twenty-four hours before harvesting 1 μCi of ^3H thymidine (specific activity 1 Ci/mM) in a volume of 0.1 ml was added to the cultures. After 24 hours the cultures were centrifuged, the sediment was washed sequentially with TC 199, concentrated acetic acid and methanol and the precipitate was dried at 37°C. Radioactivity was measured by liquid scintillation in a Packard apparatus. Incorporation of ^3H thymidine was examined 24, 48, 96 and 120 hours after cultivation was started.

To examine the delayed type skin reaction, an intracutaneous skin test was performed with an intracutaneous administration of 0.05 ml of a 1:1 000 dilution of stock solution (Institute Pasteur Paris) and checked after 48 hours. In skin tests with 2,4-dinitrochlorobenzene a 10% solution was used for sensitization and a 1:1 000 dilution of the 10% stock solution

Table 5 Chromosomal studies in ataxia telangiectasia

Patient no	Total no of counted and analysed cells	No of cells with chromosome count of				No of breaks	Breaks percent	Other structural aberrations
		<45	45	46	47			
1 Zs Sz	III	1	3	32	2	4	10	—
2 D L	30		1	29		1	3	—
3 P J	27	2	1	24		—	—	—
4 H L	15	1	2	12		1	6	—

35) As can be seen from Table 6 even one and the same individual may show different reactions if examined with different antigens. It is generally agreed that lymphoblastic transformation is the best method at present to study the cellular immunity; the procedure requires however a reliable technique. Although a decrease in the blastoid transformation was observed in all ataxic patients, it was variable. Data in this respect in the literature is shown in Table 7. Similarly to our findings it can be seen that values found in ataxic cases by the authors listed under 2, 3 and 4 reached and even exceeded the lower limit of the controls.

Differences are presumably due to errors of the morphological method (34). In ataxia telangiectasia there are numerous transitional cell forms which some authors classify as non-transformed lymphoid cells (18), some others however describe them as transformed cells.

Registration of the mitotic index seems to be much more reliable and it yielded greater difference in our cases than the morphological evaluation of blastoid transformation did even

though it has also the disadvantage of being based on sampling, i.e. it is never possible to count all the cells in the cultures. We agree with Nasputz et al. (27) that the best method is the evaluation of ^3H thymidine uptake. In our cases the latter method revealed a considerable decrease in DNA synthesis.

There was no decrease of blastoid transformation in our first case (Zs Sz, Table 2) in 1966 (37). The same patient died 2 years later in consequence of a pyloric tumor (colloid carcinoma). In the course of these 2 years the lymphocytes of the patient were cultured three more times and each examination revealed a decreased blastoid transformation of varying degree. The different results may be due to a change in the degree of inhibition of blastoid transformation but it may as easily be attributed to the inexactness of morphological evaluation.

IgA deficiency is sometimes associated with chromosomal aberrations. The chromosome 18 seems to be affected in most cases (either deletion or partial trisomy) but D/D translocation

Table 6 Delayed type skin reaction in ataxia telangiectasia

Patient no	Tuberculin reaction	PPD	Candidin (1:1000)	2-4 dinitro-chlorberzol (1:100)
1 Zs Sz	1:10 negativ	—	Negativ	Negativ
2 D L	1:100 negativ	1:1000 negativ	Negativ	±
3 P J	1:100 10:10 mm	1:1000 negativ	Negativ	±
4 H L	1:100 negativ	1:1000 negativ	Negativ	—
5 Sz S	1:10 negativ	—	Negativ	±
6 Sz G	1:1000 5:8 mm	1:1000 negativ	Negativ	+

Table 2 *Lymphoblastic transformation and mitotic index in six patients with ataxia telangiectasia^a*

Patient no	Percentage of blastic transformation		Mitotic index		IgA (serum)	Delayed type skin reactions ^b	Infections
	Patient	Control	Patient	Control			
1 Zs Sz	60.7	70.9	0.4	1.1	No	Decreased	±
2 D L	79.6	87.6	0.3	1.1	No	Decreased	0
3 P J	75.9	79.1	0.7	0.5	No	Slightly decreased	0
4 H L	75.9	87.5	0.1	0.9	Decreased	Decreased	+
5 Sz S	74.1	80.4	0.1	2.8	Normal	Decreased	±
6 Sz G	67.9	79.3	0.3	1.8	Normal	Slightly decreased	+
Mean and SD	$\bar{x} = 72.3 \pm 6.87$ 60.7-79.6	$\bar{x} = 80.8 \pm 6.23$ 70.9-87.6	$\bar{x} = 0.3 \pm 0.22$ 0.1-0.7	$\bar{x} = 1.7 \pm 1.01$ 0.5-2.9			
Ranges	$p = 0.5$		$p < 0.01$				

^a The morphological examination of blastic transformation was not made in our patient 7 because repeated culturing was not possible owing to the lack of parents' approval.

^b See Table 1.

showed a decrease in DNA synthesis in ataxic patients. In the cultures of controls ³H thymidine incorporation reached the peak after 96 hours and in ataxic subjects after 120 hours (Table 4).

Results of chromosome studies are listed in Table 5. In 1 case (Zs Sz) the number of breakages slightly exceeded the upper limit of values registered in the controls while in the other cases numerical or structural aberration breakage or signs of translocation could not be found. Findings of delayed type skin reactions are shown in Table 6.

DISCUSSION

Ataxia telangiectasia is a clinical syndrome characterized by cerebellar ataxia and oculocutaneous telangiectasia. These symptoms are associated with immunologic abnormalities which vary from patient to patient. IgA, for instance, is usually absent from plasma, although in certain cases (including some of ours) the plasma IgA concentration may be perfectly normal (2, 8, 16, 19, 24, 31, 40). The common bacterial antibody level was likewise variable (9, 13, 29, 30). As regards their de-

layed type skin reactions, patients with ataxia telangiectasia behave again variously, while the reactions are usually weak, normal reactions have also been registered (3, 16, 24, 30, 31).

Table 3 *Results of studies of lymphoblastic transformation during 2 years in patient 1*

Zs Sz	Elastic transformation	Mitotic index
1 (1966 V)	68.3	1.1
2 (1966 XI)	54.2	0.1
3 (1966 XII)	60.3	0.3
4 (1968 III)	60.2	0.1

Table 4 *Lymphoblastic transformation estimated by ³H thymidine incorporation. Counts per minute incorporated*

	Ataxia telangiectasia				Normal
	P J	T F	Zs Sz	Mean	
24 h	1 375	1 134	1 616	1 375	1 679
48 h	1 834	1 200	3 764	2 333	11 901
96 h	21 756	23 137	36 052	26 982	62 523
120 h	24 027	31 465	30 681	28 724	33 966

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Patient no	Total no of counted and analysed cells	No of cells with chromosome count of				No of breaks	Breaks percent	Other structural aberrations
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5 Sz II	1:10 negativ	—	Negativ	±
6 Sz G	1:1000 5:8 mm	1:1000 negativ	Negativ	+

Table 7 *Literary data concerning the morphological examination of lymphoblastic transformation in ataxia telangiectasia*

Patient no	Authors	No of cases	Per cent of blast cells taken the controls values to 100	Per cent of blasts	Ranges of blasts	
			Patients	Controls	Patients	Controls
1	Leikin S L et al 1966 (23)	5	37.8	74.0	20.4-46.1	63.6-84.5
2	Lévesque H et al 1966 (24)	4	39.2	75.0	0-75	75-
3	Oppenheim J J et al 1966 (28)	5	34.0	88.0	0-91	23-93
4	Hayakawa A & Kobayashi N 1967 (20)	2	55.5	80.3	34.6-76.8	74.1-91.6
5	Gotoff S P et al 1967 (17)	1	23.2	75.0	17.5-29.0	60-90
6	Gropp A & Fratz G 1967 (18)	2	22.2	89.0	20.0-23.5	88-90
7	Our patients	6	72.3	80.8	60.7-79.6	70.9-87.6

has been found too. However, numerous authors (5, 10, 12, 32, 33, 41, 43) have not found any chromosomal abnormality. Increased spontaneous chromosome breakage was observed by Jacobs et al (22) in Swiss type agammaglobulinaemia by Hecht et al (21) and by Gropp & Fratz (18) in ataxia telangiectasia. The latter finding can have a connection with the well known increased frequency of leukaemia and lymphoreticular tumours in ataxia telangiectasia (3, 6, 7, 14, 17, 21, 31). In contrast to our patients suffering from Fanconi's anaemia (38) it was only in one of the cytogenetically analysable cases of ataxia that the number of breakages slightly exceeded the control values: this was the very patient who developed malignancy (Table 5). No breakage or other chromosomal aberration was observed in any of the other subjects. Normal chromosome patterns were found also by Utian (44), Zellweger (46), Schuster et al (39) and Young et al (45) in ataxia telangiectasia. It seems that contrary to the decreased blastic transformation, increased breakage of the chromosomes is not a constant feature of this syndrome.

SUMMARY

In 6 patients suffering from ataxia telangiectasia morphological evaluation of lymphoblastic transformation was performed, in one of these cases it was repeated four times. In 3 cases the incorporation of ^3H thymidine was measured to examine the degree of the lymphoblastic transformation. Both methods revealed decreased lymphoblastic transformation though with the last method the decrease was much greater. No correlation was found between the degree of decreased blastic transformation, the infections and the plasma IgA level. Delayed type skin reactions were subnormal in varying degrees. Increased frequency of chromosome breakage was observed in 1 case—who developed malignancy and died—but no chromosomal aberrations were found in the three other cytogenetically examined patients.

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Table 7 *Literary data concerning the morphological examination of lymphoblastic transformation in ataxia telangiectasia*

Patient no	Authors	No of cases	Per cent of blast cells taken the controls values to 100	Per cent of blasts	Ranges of blasts	
			Patients	Controls	Patients	Controls
1	Leikin S L et al 1966 (23)	5	37.8	74.0	20.4-46.6	63.6-84.5
2	Lévesque H et al 1966 (24)	4	39.2	75.0	0-75	75-
3	Oppenheim J J et al 1966 (28)	5	34.0	88.0	0-91	23-93
4	Hayakawa A & Kobayashi N 1967 (20)	2	55.5	80.3	34.6-76.8	74.1-91.6
5	Gotoff M P et al 1967 (17)	1	23.2	75.0	17.5-29.0	60-90
6	Gropp A & Fratz G 1967 (18)	2	22.2	89.0	20.0-23.5	88-90
7	Our patients	6	72.3	80.8	60.7-79.1	10.9-87.6

has been found too. However, numerous authors (5, 10, 12, 32, 33, 41, 43) have not found any chromosomal abnormality. Increased spontaneous chromosome breakage was observed by Jacobs et al (22) in Swiss type agammaglobulinemia by Hecht et al (21), and by Gropp & Fratz (18) in ataxia telangiectasia. The latter finding can have a connection with the well known increased frequency of leukaemia and lymphoreticular tumours in ataxia telangiectasia (3, 6, 7, 14, 17, 21, 31). In contrast to our patients suffering from Fanconi's anaemia (38) it was only in one of the cytogenetically analysable cases of ataxia that the number of breakages slightly exceeded the control values: this was the very patient who developed malignancy (Table 5). No breakage or other chromosomal aberration was observed in any of the other subjects. Normal chromosome patterns were found also by Utian (44), Zellweger (46), Schuster et al (39) and Young et al (45) in ataxia telangiectasia. It seems that contrary to the decreased blastic transformation, increased breakage of the chromosomes is not a constant feature of this syndrome.

SUMMARY

In 6 patients suffering from ataxia telangiectasia morphological evaluation of lymphoblastic transformation was performed in one of these cases it was repeated four times. In 3 cases the incorporation of ^3H thymidine was measured to examine the degree of the lymphoblastic transformation. Both methods revealed decreased lymphoblastic transformation though with the last method the decrease was much greater. No correlation was found between the degree of decreased blastic transformation, the infections and the plasma IgA level. Delayed type skin reactions were subnormal in varying degrees. Increased frequency of chromosome breakage was observed in 1 case—who developed malignancy and died—but no chromosomal aberrations were found in the three other cytogenetically examined patients.

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THE BEHAVIOUR OF SOME INDICES OF CALCIUM PHOSPHATE METABOLISM IN TURNER'S SYNDROME

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In many individuals with Turner's syndrome osteoporotic changes may be seen in the skeleton. Theoretically these changes may be caused by a disturbance in calcium phosphate metabolism. Little data is available in the literature on this problem. It is not possible to decide on the basis of the available data to what extent these disturbances are due to the basic disease and to what degree a superimposed disorder is responsible for them. Some interesting observations made on patients with Turner's syndrome who had short metacarpal and metatarsal bones suggest that the calcium phosphate disturbances found are similar to those of pseudohypoparathyroidism (3, 15).

The aim of this study is to evaluate some indices of calcium phosphate metabolism and renal regulation of acid base balance in individuals with Turner's syndrome.

MATERIAL AND METHODS

Clinical, radiological, hormonal and cytogenetic studies were performed on a group of 11 patients with Turner's syndrome aged 10 to 18 years. In 1 patient (case 6) there were symptoms typical of rickets (hypophosphatemia, radiological signs of rickets in bones) (5).

After establishing the diagnosis, calcium and phosphate balance studies were carried out. The excretion of calcium and phosphate was determined in faeces collected for 3 consecutive days. For 3 days preceding and during the faeces collection the patients were kept on a constant diet containing 650 mg of calcium and 950 mg of phosphate per day.

The amounts of calcium and phosphate administered were calculated from the tables of their contents in foodstuffs after comparing them with the data obtained from direct determinations in Department of Human Nutrition (Poznań). Following the balance studies in intravenous calcium loading test (15 mg/kg b.w. over 4 hours) was performed (4, 13). An intravenous parathormone loading was performed modified as follows: on the first day urine was collected from 8 a.m. to 2 p.m., blood was taken at 11 a.m. On the second day 200 IU parathormone (Lilly) was administered at 8 a.m. as a 3 hour intravenous infusion; urine was collected and blood was taken as on the first day. This modification was introduced in order to avoid difficulties connected with diurnal changes in calcium and phosphorus (1).

Calcium, phosphate and creatinine concentrations in blood and urine were determined during each of the loading tests. Calcium was determined with a flame photometer, phosphate by Fiske-Subbarow method and creatinine by Folin-Wu method. The clearance of phosphate (Cp) was determined in ml/min/1.73 m² and reabsorption of phosphate in renal tubules (TRP) was calculated as a percentage of the amount filtered.

The ability of the kidney to maintain acid base balance was evaluated by ammonium chloride test as described by Greder & Guttman (2) giving orally 0.1 g NH₄Cl per kg of body weight. The excretion of titrable acidity, the ammonium ion excretion and the total excretion of hydrogen ion before and after loading were determined.

RESULTS

Clinical data and the results of radiological, cytogenetic and renal function studies are presented in Table 1. Table 2 shows indices of calcium phosphate metabolism and parameters of renal acid base balance. The results ob-

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Dilution and concentration of urine	Sex chromatin	Karyotype
001 1078	Neg	45/XO
003- 1030	Neg	45/XO
003- 1077	Neg	45/XO
007- 1077	Neg	45/XO
007 1030	Neg	—
007 1033	Neg	45/XO



Fig 1 Influence of calcium loading on phosphate excretion in Turner's syndrome shaded area normal values

cium and phosphate levels behaved normally (13) Cp was decreased considerably in 4 cases (cases 1 3 4 and 5) TRP increased distinctly only in 2 cases (cases 3 and 6) After Parathormone loading (Fig 2) no rise in serum calcium level was observed except in 1 patient (case 6) in 1 patient no decrease in serum phosphate occurred (case 1) and in 1 (case 6) a rise was observed Cp increased distinctly in 4 cases (cases 1 3 4 and 5) TRP decreased only insignificantly in 5 cases in 1 case even slightly increased (case 6)

Hydrogen ion excretion expressed either as titrable acidity or total excretion of hydrogen ion (Fig 3) was decreased both before and after NH_4Cl loading. Similarly the ammonium ion excretion was decreased in all the patients except in case 5

No exact correlation was found between the disturbances in the calcium phosphate metabolism indices studied and the magnitude of

- 3 cases in 3 cases slightly raised (cases 3 5 and 6) Tubular reabsorption of phosphate (TRP) in 3 patients approached the lowest normal values in 2 patients slightly (cases 4 and 5) and in 1 patient distinctly lowered (case 6) After calcium loading (Fig 1) the serum cal

excretion of H^+ before and after HCl in / Eq/min / 73 m^2

titrable acidity	Total excretion
37	36.8
73	58.7
47	39.3
72	57.3
21	30.5
11	69.8
46	37.4
61	81.8
100	—
31	08.1
08	30.4
23	71.5
53	103.5
87	177.3

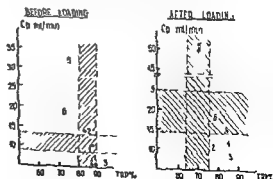


Fig 2 Influence of parathormone loading on phosphate excretion in Turner's syndrome shaded area normal values

Table 1 Clinical and laboratory data in individuals with Turner's syndrome

Case	Age in y	Height cm (deficit)	X ray of bones	Alkaline phosphatase u Bod	Ccr (ml/min/1.73 m ²)	Electrolytes in urine			
						Na (mEq/kg/24 h)	K	Ca	P (mg/1g/24 h)
1	18	146 (11)	Osteoporosis Brachydactyly IV V metatars	54	95	28	0.9	0.08	18
2	16	137 (14)	Osteoporosis Brachydactyly IV metacarp	47	75	31	0.9	0.11	15
3	15	128 (19)	Osteoporosis	57	55	33	0.7	0.07	14
4	14	135 (15°)	Osteoporosis Brachydactyly IV metatars	44	85	38	1.1	0.06	19
5	14	125 (20)	Osteoporosis	55	105	31	0.7	0.09	17
6	10	110 (25)	Osteomalacia Signs of rickets in the epiphyses	67	80	28	0.6	0.09	13
Normal values						35 ±1.3	0.9 ±0.4	0.09 ±0.04	16 ±4

tained were compared (Figs 1-3) with the values found in 10 healthy children aged 8-14 years

In all subjects studied the calcium level in blood was normal, the phosphate level was normal in 4 patients in 1 slightly lowered (case

5) and in 1 distinctly lowered (case 6) Calcium excretion in faeces exceeded the amounts ingested in 4 cases (cases 1, 2, 3 and 6) and in 2 cases was normal Phosphate excretion in faeces was normal

Clearance of phosphate (Cp) was normal in

Table 2 Indices of calcium phosphate metabolism in Turner's syndrome

Case	Balance			Calcium loading				Parathormone loading			
	Ca ()	P ()		In serum		In urine		In serum		In urine	
				Ca (mEq/l)	P (mg)	Cp (ml/min)	TRP ()	Ca (mEq/l)	P (mg)	Cp (ml/min)	TRP ()
1	-23	+62	Before	4.6	4.3	9.7	86.6	4.69	4.4	10.3	89.9
			After	6.3	4.8	7.2	90.1	4.79	4.4	14.4	86.3
2	-40	+48	Before	4.7	5.1	10.5	85.7	4.84	4.8	14.8	84.6
			After	5.4	5.8	8.2	88.1	4.84	4.3	13.8	79.9
3	-14	+52	Before	4.7	4.3	13.8	80.0	4.73	5.8	5.8	92.8
			After	6.7	5.1	10.0	92.4	4.92	4.9	8.7	85.4
4	+30	+44	Before	4.6	5.2	12.1	77.4	4.63	5.5	4.4	91.8
			After	5.6	5.9	9.1	83.6	4.80	4.4	15.8	81.0
5	+30	+66	Before	4.7	3.3	16.2	79.9	4.70	3.4	31.9	74.3
			After	4.9	4.3	11.2	74.8	4.78	2.3	52.5	71.0
6	-26	+78	Before	4.9	2.8	13.2	67.0	4.90	3.1	20.2	72.0
			After	5.9	3.8	11.4	88.5	5.50	4.4	20.5	78.8
Mean			Before	4.7	4.2	12.7	79.0	4.80	4.6	10.5	87.0
Normal values			After	5.3	4.8	6.1	91.0	5.00	3.9	24.2	73.0

activity of peripheral organs to parathyroid hormone reminiscent of the findings in pseudohypoparathyroidism may play a certain role in the pathogenesis of observed changes in the nervous system and of the deficiency of growth. The diminished ability of the kidney to excrete the excess of acid may play also a significant role in a growth failure. A positive correlation was observed between the deficit of height and the diminution of hydrogen ion excretion.

SUMMARY

The results of calcium and parathormone loading tests in 6 individuals with Turner's syndrome indicate a certain degree of insensitivity of the tissues especially of the kidney when compared with healthy individuals. A similar insensitiveness expressing itself by a decrease in hydrogen ion excretion was found after ammonium chloride loading. An analysis of the results suggest the existence in this syndrome of insensitiveness of renal tubules to regulatory factors. Osteoporosis and stunting of growth may be connected partly with intestinal calcium loss occurring at puberty and partly with this insensitiveness to regulatory factors.

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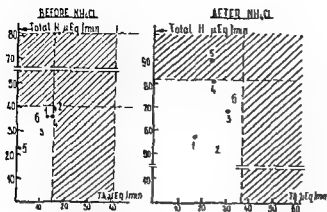


Fig 3 Hydrogen ion excretion in Turner's syndrome
▨ normal values

growth deficiency Neither was it possible to show any precise correlation between the degree of osteoporosis and the presence of other bony changes such as shortening of metacarpal and metatarsal bones in the cases 1, 2, 4, and the variations of the indices of calcium-phosphate metabolism. Similarly no correlation was observed between the increased calcium excretion in faeces and the abnormal results of load tests. The result obtained suggest only a certain correlation between the age and the increased calcium excretion in faeces in the oldest girls (cases 1, 2 and 3). An increased calcium excretion in faeces in the youngest girl (case 6) may be connected, at least in part to the disturbances associated with rickets (14).

There was, however, sufficiently marked positive correlation between the rate of reduction of hydrogen ion excretion expressed both as titrable acidity and total excretion and the degree of growth deficiency.

DISCUSSION

The nature of the disturbances in calcium phosphate metabolism in Turner's syndrome has not as yet been elucidated.

In the present investigations, the calcium and phosphate levels in blood were normal. The pronounced hypophosphatemia in case 6 is probably due to coexisting rickets (5). Virtually no changes were observed in C_p , TRP, or phosphate excretion in urine, the only ex-

ception being a very low degree of TRP in case 6 mentioned above.

The calcium loading tests were in part in conformity with the results obtained in normal subjects (4, 13). In all the cases there was a rise in calcium and phosphate levels in blood. In 4 cases there was a distinct decrease in C_p . But in only 2 cases was a more pronounced increase in TRP found (Fig 3). The parathyroid hormone loading revealed a diminished end organ sensitivity to this hormone, especially as expressed in terms of alterations of TRP.

The reason for the above mentioned disturbances in calcium-phosphate metabolism indices is still obscure. There may be a diminished reactivity of peripheral tissues to regulatory factors, especially to parathyroid hormone. Changes of this kind may affect growth and skeletal formation process as, on one hand, they diminish the transport of phosphate to cell (9, 11, 12), and, on the other hand, they reduce the exchange of calcium and phosphate within the skeleton (8, 10).

The investigation on calcium balance revealed an increased calcium excretion in faeces in the oldest subjects studied. The attempts to find the cause of this phenomenon were unsuccessful. This negative calcium balance in Turner's syndrome, however, may be one of the causes making pubertal growth acceleration impossible.

Abnormal kidney function was observed while investigating its role in regulation of the acid base balance. The decreased hydrogen ion excretion expressed as titrable acidity found before and after ammonium chloride loading proves some impairment of efficient work of nephron in this respect. Ammonium ion excretion although not shown in Table 2 was also low except for case 5 (hence a higher total hydrogen ion excretion in that case). Other disturbances in renal function have previously been observed in the cases of Turner's syndrome (6, 7).

On the basis of our investigations the function of parathyroid glands in the Turner's syndrome appears to be normal. The changed re-

Table 1 Patient data for three children on regular haemodialysis treatment

Patient	Age at the start (years)	Body weight at the start (kg)	Duration of treatment (months)	No of treatments	Diagnosis
1	14	25.5	25	221	Chronic pyelonephritis vesico-ureteral reflux
2	13	32.5	15	131	Chronic pyelonephritis vesico-ureteral reflux
3	7	18.0	2	16	Hereditary renal disease congenital nephrosis

sexual development with normal menstruations has occurred

Prior to dialysis only patient 2 was hypertensive during treatment all are normotensive without drugs. Patients 1 and 2 were nephrectomized for control of urinary tract infection.

None of the patients had clinical signs of neuropathy. Signs of hyperparathyroidism or other abnormality in calcium metabolism have not been noted.

An arteriovenous fistula may cause overgrowth of the affected limb if the metaphyses are not closed. This phenomenon is a classical part of the syndrome of Klippel-Trenauney and Parkes-Weber. The present patients displayed no signs of hypertrophy or increased skeletal growth on physical or roentgenological examination possibly due to the fact that the fistula is made from a very distal artery to a superficial vein. There were no clinical signs of insufficient nutritive blood flow to the fistula arm.

In patient 1 cardiac output, central venous pressure and arterial pressure with open and closed fistula were measured as well as the

total blood volume (Table 2). The difference in cardiac output with open and closed fistula was less than 10%. We could find no signs of adverse effect upon the heart and the blood volume was within normal limits. ECG and chest X-ray examinations have shown no indication of cardiac decompensation.

All the patients are emotionally well adjusted to dialysis treatment. Their general condition has much improved and the repeated percutaneous cannulations have given them no emotional problems. Patient 1 has now finished her schooling and is working full time; the days she is not on dialysis. Patient 2 has returned to school between the dialyses. They have been able to use their arms without restrictions and they do not care about the presence of a fistula.

We have found the Cimino-Brescia subcutaneous direct arteriovenous fistula well suited for haemodialysis in children of this age. We have found no signs of circulatory overload during the time of observation and the risk of overgrowth of the arm carrying the fistula is probably overrated.

Table 2 Haemodynamic data for patient 1

Length 144 cm weight 8 kg blood volume 2.5 l haematocrit 26

	Cardiac output (l/min)	Arterial pressure (mmHg)	Central venous pressure (mmHg)
Fistula open	6.7	130/70	~1
Fistula closed	6.3	133/75	~2

SUMMARY

The subcutaneous fistula according to Cimino-Brescia was used in three children 14, 13 and 7 years of age for regular haemodialysis up to 25 months. No adverse effects on the central or local circulation were recorded and no significant difference appeared in the growth of the fistula limb compared with that of the contralateral. The repeated percutaneous punc-

ARTERIOVENOUS FISTULA FOR HAEMODIALYSIS IN CHILDREN

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Regular haemodialysis is getting more and more common even in children, most of whom are prepared for kidney transplantation. In most cases reported, access to the patient's vascular system has been given by an external arteriovenous shunt according to Quinton et al (11) using a prosthesis of teflon and silastic (3, 5, 6, 7, 9, 12, 13). Distal arteries, e.g. the radial artery or posterior tibial artery, have been cannulated in older children (> 30 kg) the brachial, axillary or femoral arteries in smaller children and infants. This external type of shunt has some disadvantages: risk of infections, coagulations, skin necrosis, bleedings etc., and the patients are incapacitated by its presence.

Arteriovenous fistula as described by Brescia et al (4) is often used in the adult and has eliminated many of the disadvantages of the Quinton-Scribner technique. In children the subcutaneous fistula has been avoided due to the supposed risk of complications with cardiac overload and hypertrophy of the limb carrying the fistula (5, 12).

We have tried the subcutaneous fistula in three children on regular haemodialysis.

METHODS

Surgical technique. We have used the technique described by Brescia et al (4) with some modifications (10). The anastomosis was made side to side between the radial artery and the cephalic vein on the non-

dominant left arm and all four limbs of the vessels were left open.

Dialysis technique. Metal cannulas gauge 14-16 were used for puncture of the veins and were connected to the Alwall Gambro disposable dialyzer (1, 2) using a blood pump. In the two older children the normal 11-layered model for adults was used but the smallest child was treated with a 6-layered model (8). Treatments were given 4-8 hours twice weekly.

Roentgen examinations were performed on the chest (heart size, overhydration) and both forearms (length of radius and ulna, bone thickening or sclerosis).

CASE MATERIAL

Three children, 14, 13 and 7 years of age, had chronic renal failure with endogenous creatinine clearance values less than 5 ml per minute per 1.73 m² and were selected for regular haemodialysis in preparation for renal homotransplantation (see Table 1).

RESULTS AND DISCUSSION

The fistula veins were used for dialysis 2 days after operation in patient 1. In the other two patients we made the fistulae weeks before dialysis treatment was necessary. Cannulations of the veins were made by dialysis nurses and have given very slight technical problems. No major haematoma or local infection occurred at the site of the cannulations.

All the patients were underweight and smaller than normal children when dialysis treatment was started. The two elder patients in whom treatment has continued long enough for evaluation have both grown, and in patient 2

REVIEW ARTICLE

ARTERIAL ANOMALIES CAUSING COMPRESSION OF
THE TRACHEA AND/OR THE OESOPHAGUS

A Report of 30 Symptomatic Cases

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Anomalies of the aortic arch and its branches are fairly common (6-12). Most of these anomalies are asymptomatic. They are diagnosed at autopsy by mere chance with radiological examination of the trachea and oesophagus or in angiocardiology for other congenital cardiovascular malformations. Symptomatic cases are rare, the symptoms being caused by compression of the trachea and/or the oesophagus. Symptoms may arise from anomalies not only of the aorta and its branches but also from an aberrant left pulmonary artery or from an aneurysm of the pulmonary artery (5). In our experience the prevalence of symptomatic cases among other congenital cardiovascular malformations is less than 0.5%. The symptoms caused by arterial derangements within the chest may be life threatening and therefore early diagnosis and surgical treatment are important in these symptomatic cases.

The literature on these anomalies is considerable including aspects on embryology and anatomy, symptoms, diagnosis and surgery (2, 4-14). The aspects on the diagnostic aids and surgery have however been somewhat contradictory and it is the aim of this cooperative

study to evaluate the diagnosis and treatment of arterial anomalies with such severe symptoms from compression of trachea and/or oesophagus that surgery has been indicated and to report the long term postoperative results.

There are many different types of vascular rings and other arterial anomalies causing similar symptoms and there is a great variability within the special group. The following classification is confined only to those anomalies found in our material. They do however constitute the most common types (see the schematic figure Fig. 1).

1 *Double aortic arch* The two arches encircle the trachea and the oesophagus.

2 *Right aortic arch with left ligamentum arteriosum or patent ductus arteriosus* The ligamentum or the ductus completes the ring by passing from the left side behind the oesophagus to the aorta.

3 *Aberrant right subclavian artery (arteria lusoria)* The vessel arises as the last branch from the aortic arch and passes to the right side of the chest usually behind the oesophagus but may also pass between the oesophagus and the trachea or in front of the trachea.

tures have given no significant technical or psychological problems to the patients nor to the nurses

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Table 1 Vascular Ring cases of aortic arch

Case no	First symptom age	Indication for surgery	Operat n		Surgical procedure & vision of	Postoperative course	Additional anomalies
			Age	Year			
1	1 mo	Constant stridor, recurrent resp infect	5 y	1950	Anterior arch	Uneventful	
2	1 wk	Increasing stridor, recurrent resp infect	18 mo	1951	Posterior arch + PDA	Stridor first months	
3	1 wk	Constant stridor	2 mo	1950	Ant arch + left subclav artery	Tracheostomy Decannulation after 18 mo	
4	1 wk	Increasing stridor	2 mo	1954	Post arch + lig art	Tracheostomy died 1 wk after operation	
5	1 wk	Increasing stridor + recurrent resp infect	6 mo	1955	Post arch + lig art	Cyanotic attacks for 1 mo after operation	
6	1 wk	Increasing stridor	3 mo	1958	Ant arch + PDA	Tracheostomy + respirator Died 4 wk after operation	Atrial septal defect primum type
7	3 mo	Increasing stridor	3 mo	1961	Post arch + lig art	Uneventful	
8	1 wk	—	10 days	1958	Ant arch	Tracheostomy + respirator Died 2 days after operation	Tracheo oesophageal fistula
9	1 wk	Stridor + cyanotic attack with unconsciousness	6 mo	1959	Post arch	Tracheostomy + respirator	
10	1 wk	Increasing stridor	6 mo	1959	Left common carot art	Tracheostomy + respirator Died 14 days after operation	
11	1 mo	Dysphagia + stridor	4 mo	1965	Ant arch	Stridor for 1 year after op	
12	1 wk	Reflex apnea	13 mo	1967	Ant arch + lig art	Tracheostomy + respirator 1 week	
13	1 mo	Increasing stridor	18 mo	1968	Post arch + lig art	Uneventful	
14	1 wk	Increasing stridor	6 mo	1967	Post arch + PDA	Tracheostomy + respirator Decannulation 18 mo after operation Slight stridor 3 y after operation	

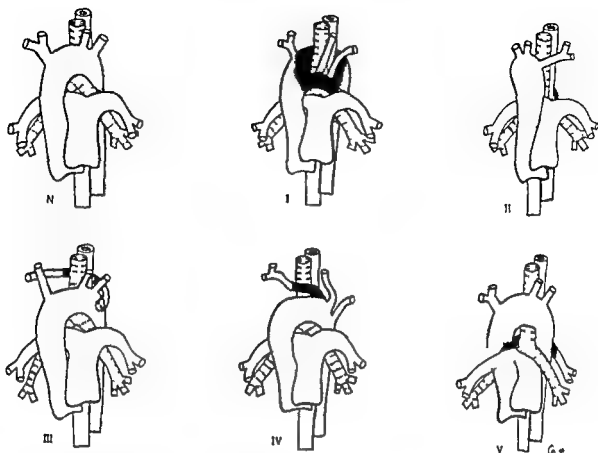


Fig 1 Schematic drawings showing the various vascular anomalies. Black areas show the compressing part of the vessels. N normal anatomy. I double aortic arch. II right aortic arch with left ligamentum

arteriosum. III aberrant right subclavian artery. IV anomalous innominate artery. V anomalous left pulmonary artery.

4 Anomalous innominate artery. The artery originates from the aortic arch distally and passes in front of the trachea to the right side of the neck.

5 Anomalous left pulmonary artery. The bifurcation of the pulmonary artery is displaced to the right and the left pulmonary artery passes posteriorly turning around the right superior tracheobronchial angle to the left lung between the oesophagus and the trachea.

CLINICAL MATERIAL

The material consists of 30 cases: 16 boys and 14 girls which were seen during a period of 19 years from 1950 to 1968. Five cases have previously been reported (2, 3). The individual data are given in Tables 1-5. Double aortic arch was present in 14 cases, right aortic arch with left ligamentum arteriosum in 5

aberrant right subclavian artery in 4, anomalous innominate artery in 4 and anomalous left pulmonary artery in 3 cases respectively. Associated cardiovascular malformations were diagnosed in 7 patients and other significant malformations were diagnosed in another 5 cases. Two infants had oesophageal atresia and another case had tracheo-oesophageal fistula. Anal atresia and recto-urethral fistula was observed in one child with anomalous pulmonary artery. Three children with arterial lusoria were mentally retarded, one of them had Down's syndrome.

SIGNS AND SYMPTOMS

In all children the symptoms which could be referred to the vascular anomaly started before the age of 5 months. In 18 cases the onset of symptoms occurred already during the first week of life. Ten out of these 18 cases had double aortic arch (Table 1). *Stridor* and other

Table 1 Vascular Ring cases double aortic arch

Case no	First symptom	Indication for surgery	Operation		Surgical procedure division of	Postoperative course	Additional anomalies
			Age	Year			
1	1 mo	Constant stridor + recurrent resp infect	5 y	1950	Anterior arch	Uneventful	
2	1 wk	Increasing stridor + recurrent resp infect	18 mo	1951	Posterior arch + PDA	Stridor first months	
3	1 wk	Constant stridor	2 mo	1950	Ant arch + left sub lary artery	Tracheostomy Decannulation after 18 mo	
4	1 wk	Increasing stridor	2 mo	1954	Post arch + lig art	Tracheostomy died 1 wk after operation	
5	1 wk	Increasing stridor + recurrent resp infect	6 mo	1955	Post arch + lig art	Cyanotic attacks for 1 mo after operation	
6	1 wk	Increasing stridor	3 mo	1958	Ant arch + PDA	Tracheostomy + respirator Died 4 wk after operation	Atrial septal defect primum type
7	3 mo	Increasing stridor	3 mo	1961	Post arch + lig art	Uneventful	
8	1 wk	Increasing stridor	10 days	1958	Ant arch	Tracheostomy + respirator Died 2 days after operation	Tracheo-oesophageal fistula
9	1 wk	Stridor + cyanotic attack with unconsciousness	6 mo	1959	Post arch	Tracheostomy + respirator	
10	1 wk	Increasing stridor	6 mo	1959	Left omoron carot art	Tracheostomy + respirator Died 24 days after operation	
11	1 mo	Dysphagia + stridor	4 mo	1965	Ant arch	Stridor for 1 year after op	
12	1 wk	Reflex apnoea	13 mo	1967	Ant arch + lig art	Tracheostomy + respirator 1 week	
13	1 mo	Increasing stridor	18 mo	1968	Post arch + lig art	Uneventful	
14	1 wk	Increasing stridor	6 mo	1967	Post arch + PDA	Tracheostomy + respirator Decannulation 18 mo after operation Slight stridor 3 y after operation	

Table 2 Vascular Ring cases Right aortic arch with left ligamentum arteriosum or persistent ductus arteriosus

Case no	First symptom age	Indication for surgery	Operation		Surgical procedure division of	Postoperative course	Additional anomalies
			Age	Year			
15	1 wk	Recurrent respir infection	16 mo	1954	Lig art	Uneventful	
16	1 mo	Attacks of cyanosis	7 mo	1955	PDA + left subclavian	Attacks of cyanosis Died 1 wk after op	Ventricular septal defect Malformed tricuspid valves
17	3 mo	Dysphagia + stridor	4 mo	1959	PDA	Uneventful	
18	4 mo	Increasing dysphagia + attack of cyanosis	18 mo	1962	Lig art	Uneventful	
19	1 wk	Recurrent respir infection	18 mo	1964	Lig art	Uneventful	Atrial septal defect primum type

Table 3 Vascular Ring cases anomalous right subclavian artery (arteria lusoria)

Case no	First symptom age	Indication for surgery	Operation		Surgical procedure division of	Postoperative course	Additional anomalies
			Age	Year			
20	1 mo	Dysphagia + convulsions of unknown etiology	3 mo	1955	Art lusoria + PDA	Uneventful	Mental retardation + PDA
21	1 wk	Dysphagia	4 y	1967	Art lusoria	Uneventful	Mental retardation
22	1 wk	Respir infections + dysphagia	6 mo	1966	Art lusoria	Uneventful	Down's syndrome
23	1 mo	Dysphagia + recurrent respir infections + stridor	8 mo	1968	Art lusoria + PDA	Uneventful	PDA + slight valvular pulmonary stenosis

Table 4. *Fascicular Ring cases anomalous innominate artery*

Case no	First symptom age	Indication for surgery	Operate at		Surgical procedure division of	Postoperative course	Additional anomalies
			Age	Year			
24	5 mo	Stridor	8 mo	1955	Innominate artery	Uneventful	Oesophageal atresia earlier operated
25	1 wk	Recurrent broncho pneumonia	7 mo	1964	Fixation of the innominate artery to sternum	Uneventful	
6	1 wk	Stridor recurrent resp infection	5 mo	1965	Fixation of the innominate artery to sternum	Stridor first weeks	Oesophageal atresia earlier operated
27	1 wk	Recurrent broncho pneumonia	6 mo	1968	Fixation of the innominate artery to sternum	Stridor a few weeks	

Table 5. *Vascular Ring cases aberrant left pulmonary artery*

Case no	First symptom age	Indication for surgery	Operation		Surgical procedure division of	Postoperative course	Additional anomalies
			Age	Year			
28	3 wk	Increasing stridor + attacks of cyanosis	5 mo	1966	Transposition of left pulmonary art	Died 1 day after operation	Anal atresia + recto urethral fistula earlier operated
29	1 wk	—	—	1967	—	—	Aphtosis of left pulm inf lobe Respiration 1 week before death
30	2 mo	Attacks of cyanosis + respir insufficiency Emphysema	5 mo	1968	Transposition of left pulmonary art	Tracheostomy + respirator Decannulation after 8 mo Reoperation after 3 mo (thrombosis left pulm art)	

respiratory difficulties were observed in all but 2 cases. The stridor was usually of inspiratory type accompanied by expiratory wheezing. Belly cough and hoarse voice were common findings. Tachypnea and other signs of respiratory distress usually occurred. The respiratory difficulties were accentuated during increased activity such as feeding and crying. Recurrent respiratory infections were prominent symptoms in 20 cases of all types. During these infections the respiratory difficulties were aggravated to such an extent that in several cases an emergency operation was performed. Eleven patients had dysphagia. This symptom was not present in any of the cases with anomalous innominate or anomalous left pulmonary artery. Attacks of cyanosis occurred in all the different types and was followed by episodes of unconsciousness in 2 cases with double aortic arch.

In most cases the diagnosis was made probable or confirmed by means of roentgenological examination of the chest including contrast filling of the oesophagus. In 8 cases the diagnosis was not disclosed at the first roentgenological examination of the chest. In 5 cases this could be explained by the fact that contrast filling of the oesophagus had not been performed and in 3 cases, an inadequate examination could explain the lack of precision in the diagnosis. Angiography was performed in 17 cases to confirm the diagnosis and to clarify the anatomy.

The roentgenological findings in double aortic arch and right aortic arch with left ligamentum arteriosum or patent ductus arteriosus were an indentation and dislocation of the oesophagus and the trachea usually at the level of the third or fourth thoracic vertebra.

The cases with aberrant right subclavian artery showed a posterior defect of the oesophagus and no tracheal compression.

The roentgenological findings in anomalous innominate artery were visible in the lateral projection with an indentation in the anterior wall of the trachea just below the suprasternal notch. The appearance of the oesophagus was normal.

The findings in anomalous left pulmonary

artery were a displacement of the lower part of the trachea to the left and forward, and an indentation on the right main bronchus near its origin. There was also an indentation in the anterior wall of the oesophagus at the level of the carina and an increased distance between the oesophagus and the trachea.

OPERATIVE TREATMENT AND RESULT

Twenty-nine cases were operated. In double aortic arch the smaller arch was divided. In 11 cases the ligamentum arteriosum was cut as well and in 1 case, the left subclavian artery was divided. In right aortic arch a patent ductus arteriosus was divided in 3 cases and ligamentum arteriosum in 2. In all cases of arteria lusoria the artery was divided and, in 2 cases a patent ductus arteriosus as well. The anomalous innominate artery was divided in 1 case and sutured to the inside of the sternum in the other 3 cases. Finally, in 2 cases, the anomalous pulmonary artery was divided and anastomosed to the main pulmonary artery or to the left branch. The third case of anomalous pulmonary artery (case 29) was not operated. This girl had respiratory difficulties from birth. As the symptoms were moderate and the right lung roentgenologically normal, surgical intervention was not considered necessary for the moment. At the age of 4 months she caught a respiratory tract infection with severe symptoms. She was admitted for operation but was in such a bad condition that only tracheostomy could be performed. During this procedure an extensive tracheal stenosis was found. She did not improve and died a week later in a respirator.

Postoperative complications were common in patients with double aortic arch and occurred in both patients with anomalous left pulmonary artery. Persisting and even increased stridor and excessive tracheo-bronchial secretions made tracheostomy necessary in 10 cases and 9 out of these were treated with respirator. Four of these 10 cases survived after respirator treatment and decannulation could be performed in

1 case after a few days and in the three other not earlier than between 9 and 18 months after the operation

There were 8 deaths including the one not operated upon. In the two children with anomalous left pulmonary artery autopsy showed a generalized tracheal stenosis. Five children with double aortic arch died postoperatively. All of them were tracheostomized and treated in respirator. The left common carotid artery instead of the anterior arch was accidentally divided in case 10. One boy no. 4 who had tracheal infection died after 1 week. Malformed tricuspid valves with signs of severe incompetence were found in case 16.

It should be noted that all postoperative deaths except one occurred during the first period of time 1950-1959. In the series of 15 cases operated between 1960 and 1968 only one child died after operation.

Information of the postoperative course has been collected in all cases. Most of the patients have been investigated with roentgenological examination of the chest including a contrast filling of the oesophagus. The postoperative follow up time varies between 9 months and 19 years.

In 7 patients the symptoms disappeared immediately or during the first month following surgery. Mild stridor or dysphagia was present in 6 patients during a period up to 5 years after operation. Two children had repeated episodes of bronchitis 1-2 years after surgery. A mentally retarded girl no. 20 died 1 year after a successful operation of arterial ligation. The cause of death was bronchopneumonia.

A mild compression and dislocation of the trachea was present in 4 patients years after operation and slight dislocation of the oesophagus was seen as long as 8 years postoperatively.

DISCUSSION

Many conditions may cause stridor in early infancy and have to be considered in the differential diagnosis of vascular anomalies. The stridor in arterial anomalies may not be ap-

parent until the age of several months and therefore not only the congenital conditions but also the acquired have to be included in the diagnostic considerations. The most common congenital stridor is probably caused by soft tracheal wall or a flaccid epiglottis. This condition as well as other structural anomalies of the larynx and the trachea may be verified by means of laryngoscopy. Macroglossia, micrognathia, thyroid cysts or ectopic struma, laryngitis, bronchitis, foreign bodies, tumors and metabolic disorders associated with hypocalcemia are other disorders which could give stridor like symptoms. In all these conditions the oesophagogram is normal. Contrast filling of the oesophagus is the main diagnostic aid to the diagnosis of double aortic arch, right aortic arch with left ligamentum aberrant, right subclavian artery and anomalous left pulmonary artery. Roentgenographic examination of the trachea gives an important contribution to the diagnosis of the last mentioned condition and of anomalous innominate artery. The roentgenological appearance could however be less apparent in early infancy and therefore repeated examination could be necessary before the diagnosis is settled. From surgical point of view angiography may be indicated giving more anatomical details especially when associated congenital heart lesions are suspected. Lobar emphysema may also be suspected as in one of our cases of anomalous left pulmonary artery. The emphysema comprised the whole right lung, however which is not seen in lobar emphysema. Bronchoscopy and tracheography seems to be of comparatively little diagnostic value. These investigations are dangerous in infants with respiratory difficulties and should in our opinion be avoided.

The respiratory symptoms in the material published here have been referred to the vascular anomaly. In a few cases with associated lesions however it may seem to be difficult to say definitely that the vascular anomaly was the main cause of the symptoms. Mental retardation, associated cardiac malformations and conditions after operation for oesophageal

atresia could give symptoms similar to those of vascular anomalies. After studying the hospital records, we still believe that the vascular malformation has been the main cause of the respiratory difficulties except in one or two questionable cases.

The operative risk should be weighed against the natural history of the disease and the post operative results. The follow-up study of this material shows that the long term results are excellent even if the improvement may take years in a few cases. Mustard et al (10) reported a material of 23 children with symptomatic vascular compression who were followed for 6 months to 7 years. During that time 2 children died because of the vascular anomaly. Twelve children, however, were asymptomatic at the time of follow up. A high mortality was reported in cases with aberrant left pulmonary artery (9). Out of 44 cases, 16 unoperated symptomatic children died and only 6 were asymptomatic. Double aortic arch and anomalous left pulmonary artery are most apt to give symptoms demanding surgery. Right aortic arch with left ligamentum arteriosum often needs operation while surgery is very seldom needed in anomalous innominate artery and in arteria lusoria. Thus, even if the symptoms in some cases may subside children with symptomatic vascular compression run a great risk and if surgery is postponed they must be kept under close observation and surgery should be seriously and repeatedly considered.

The aim of the operation is to relieve the compression. The operative technique varies from case to case as the details of the malformation are never identical.

An interesting and controversial detail in the surgical procedure is how far the dissection should be carried out around the trachea and the oesophagus.

According to Mustard et al (10) the dissection should be minimal because the soft trachea needs all supporting tissue in order not to collapse. Gross (4) on the other hand speaks in favour of an extensive dissection to remove all sheaths and fibrous band that accompany

the vessel. Lincoln et al (7) who recently published a series of 29 cases describe that 'Dissection around the trachea and oesophagus was made in all cases to divide all constricting fascial bands. No untoward effects of such a procedure are mentioned. Our series does not give support to one or the other opinion. We think that fibrous rings and sheaths should be divided with minimal dissection.

In children with double aortic arch and with anomalous left pulmonary artery the postoperative course is rarely uneventful. Difficulties are encountered especially in children with early symptoms. Out of 10 children with double aortic arch and with symptoms during the first week of life, 8 needed tracheostomy and respirator treatment postoperatively and 5 in the early series died. The complications in these children were not correlated to the age at operation.

From the material presented here, it is evident that the operative risk has been greatly reduced during the most recent years. This is explained by increased experience in the surgical technique and by improved postoperative care. The relatively low operative mortality of today is a strong argument in favour of surgical treatment of arterial anomalies causing compression of the trachea and the oesophagus. These anomalies may be life threatening and therefore early diagnosis is imperative. The presence of persistent congenital stridor should warrant radiological investigation of the trachea and the oesophagus.

SUMMARY

The experiences of diagnosis and treatment of arterial anomalies causing compression of the trachea and/or the oesophagus in 30 symptomatic cases are reported. The main diagnostic aid is the radiological examination of the chest in the anterior posterior and lateral projection including adequate contrast filling of the oesophagus. This investigation, using water-soluble contrast media, should be performed in infants with stridor and other respiratory difficulties.

and in dysphagia. With improved surgical technique and postoperative care the operative risk of today is low and surgery should be seriously considered in symptomatic cases. Operation may be necessary as an emergency treatment.

Addendum. Another nine infants have been operated with no mortality since this material was collected. Three cases had double aortic arch, five right aortic arch with left ligamentum arteriosum and one infant had anomalous innominate artery.

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SHORT COMMUNICATION

PERITONEAL DIALYSIS IN THE TREATMENT OF HYALINE MEMBRANE DISEASE OF NEWBORN PREMATURE INFANTS

Results of a Controlled Trial Preliminary Report

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In our previous animal experiments (3) we found a markedly increased tolerance against otherwise lethal hypoxia in newborn rabbits and adult mice treated by peritoneal dialysis (PD). These results can be considered as supporting data on the pathologic effect of metabolites accumulated in the body fluids as additional humoral factors inhibiting the life of the cells. On the other hand on the basis of these experiments we regarded as permissible to carry out therapeutic experiments in the cases of hyaline membrane disease (HMD) of newborn premature infants which failed to show improvement with the alkali glucose treatment.

In such cases the PD has therefore been employed in an attempt to achieve the following therapeutic effects: 1) correction of metabolic disturbances, including metabolic acidosis, without the administration of excessive quantities of fluids and electrolytes and 2) elimination of possible toxic metabolic products that may accumulate during hypoxia.

MATERIAL AND METHODS

A total of 92 premature infants with HMD have been studied: 50 received PD (dialysis group) and 42 were treated by conventional methods (alkali glucose infusion according to Usher (5)) the dose of

bicarbonate adjusted to the results of pH and BE values of arterialized capillary blood determined by Astrup's method (1) (control group).

An infant was included in the series only when it failed to improve during treatment with alkali and glucose (1) for 4 or more hours when its clinical condition was classified according to Weissers criteria (6) as grade III or 2) for 8 or more hours when its condition was classified as grade II.

Inclusion in either the dialysis or control groups was then decided by drawing lots. Conventional therapy was continued in the control groups and PD was instituted in the dialysis group by simultaneous discontinuation of any other treatment.

Intermittent PD was performed in our first 38 cases. The dialysis fluid contained 95 mEq Na⁺ 4.0 mEq K⁺ 4.0 mEq Ca⁺⁺ 2.0 mEq Mg⁺⁺ 105 mEq Cl⁻ and 15 glucose per litre. Depending on the severity of the acidosis 40 to 50 mEq/l of sodium bicarbonate was added to the solution immediately before its use. The solution contained penicillin (100 000 IU/l) but no heparin.

Continuous PD was introduced beginning with the case 39 when a suitable method had been devised. The apparatus for continuous PD is shown in Fig 1. Since the introduction of the continuous PD 100 IU heparin per litre has also been added to the solution. From this time on the exchange of dialysis fluid with a rate of 120-150 ml/hour has usually been undisturbed and the outflow only rarely impeded. The dialysis was maintained for 36 to 48 hours depending on the pattern of clinical symptoms. In a single case it lasted for 72 hours.

For better care of PD treated newborn before and during the dialysis the following parameters were controlled: bodyweight serum Na and K concentrations total serum proteins chest roentgenogram

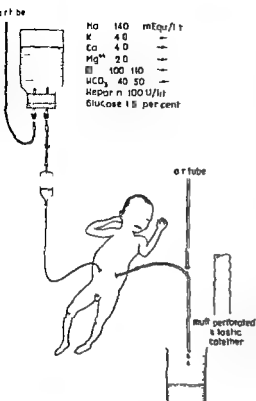


Fig 1 Apparatus for the continuous method of PD

Standard bicarbonate pH Pco₂ of the arterialized capillary blood was monitored according to Astrup (1) twice daily. Measurements directly concerning PD (abdomen circumference volume in-out) were performed. Bilirubin and protein concentration of the outflowing fluid were also measured.

The χ^2 method was applied for the calculation of difference between the mortality rates of the two groups. To determine the significance of survival curves Wilcoxon's rank sum test (4) was used.

RESULTS

The birthweights severity of HMD symptoms and blood pH values at admission were comparable in the two study groups (Table I). The survival curves of both groups during the first 5 days of life are given in Fig 2. More infants in the PD group survived the fifth day than in the control group: 21 out of 50 infants and 7 out of 42 respectively.

Clinical and laboratory observations PD was well tolerated by the premature infants

and no serious complications were observed. In particular respiratory embarrassment was not noted. Besides the hindered outflow of the dialysis fluid (only by the intermittent method) transitory abdominal distension was observed in 4 cases. In some cases oedema of the genitalia appeared but subsided soon after the end of the treatment.

The first signs of clinical improvement were observed as early as 4 hours after PD was begun and marked improvement was usually present after 24 hours. Acid base values were in most cases normalized within 12 hours. The bodyweight of the infants fell only slightly but regularly during the treatment.

The outflowing dialysis fluid was usually markedly yellow but its bilirubin content did not surpass 1 mg/100 ml. The protein content of the fluid fluctuated within wide limits and the total protein loss amounted to 1 to 2 g daily.

An autopsy was performed in each fatal case. No signs of peritoneal inflammation were noted.

Table I Distribution and mortality rate according to prognostic factors in infants in the trial

	Dialysed		Probability P	Controls	
	No	Died		No	Died
Birthweight kg					
<1.00	6	5	NS	6	5
1.00-1.50	19	13	NS	17	16
1.50-2.00	22	10	<0.05	16	12
2.00 <	3	1	NS	3	3
Severity of HMD on admission according to Wenzel's score					
0-I	7	1	<0.05	10	6
II	26	14	<0.01	24	21
III	17	14	NS	8	11
Blood pH on admission					
<7.00	5	4	NS	3	3
7.00-7.20	20	14	<0.05	19	18
7.20 <	25	11	NS	20	14
Total	50	29	<0.02	42	35

* NS—not significant

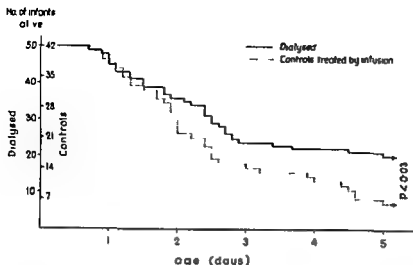


Fig 2 Survival curve in the 5 days of life of HMD of prematures treated by peritoneal dialysis and by alkali glucose treated controls

GENERAL CONCLUSION AND PLANNED FURTHER STUDIES

On the basis of our present clinical experimental series we may state that PD can be performed on premature infants without any remarkable difficulty. The method of continuous dialysis does not appear to cause any serious stress to the infants.

We are now striving to initiate PD when possible, at an earlier phase in HMD in premature infants.¹ However, more studies are necessary to determine the value of PD in the treatment of HMD in premature infants. Further clinical experiments are planned to investigate the therapeutic effects of PD by the control of arterial oxygen tension measurements.

SUMMARY

Intermittent or continuous PD has been employed in the treatment of 50 selected cases of HMD of premature infants. Twenty one infants treated by this method recovered where as only 7 of 42 survived in a comparable control group treated with alkali and glucose.

¹ Results of these studies will be published in *Acta Paediatr Scand* 11 No 3-4 1970

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Key words Respiratory distress syndrome of prematures peritoneal dialysis

CASE REPORT

TRISOMY 18 WITH OVARIAN DYSGENESIS

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Trisomy 18 was first described by Edwards et al in 1960 (3). It was then thought to be a rare syndrome but more than 200 cases have been published since. New anomalies with varying frequency have been added to those initially described and the clinical picture has become more complex. Furthermore cases with the clinical characteristics of trisomy 18 were found to have a normal karyotype (6, 9). This has a special interest and has also been observed in other syndromes as in the male Turner syndrome.

The purpose of this report is to present a case of trisomy 18 with ovarian dysgenesis and some additional uncommon pathological findings.

CASE REPORT

Female infant born by normal delivery at Alexandra Maternity Hospital at 43 weeks of gestation. Birth was uneventful. She was the first child of a 25 years old epileptic mother who from the 5th month of gestation was on diphenylantoin sodium 100 mg and phenobarbitone 50 mg daily. The father 31 years old, was healthy.

The infant's birth weight was 1980 g and the body length 46 cm. She presented multiple congenital abnormalities (Figs. 1, 2, 3 and 4): (a) odd-shaped head with prominent occiput and narrow forehead; (b) broad and flat bridged nose; (c) low set ears with

abnormal contours and a narrow external meatus; (d) low hair line; (e) small eyes; (f) small mouth with hypoplastic mandible and high arched palate; (g) upper extremities held at chest level with permanently clenched fingers and characteristic overlapping of the index over the middle and the small over the ring; (h) hypoplastic thenar and hypothenar; (i) small narrow pelvis; (j) short webbed toes.

On the second day of life she developed peri-oral cyanosis, dyspnoea and many moist rales on both lungs. The heart rate was 180 per minute. Chest X-ray revealed a globular enlarged heart. Her condition gradually deteriorated, she developed generalized cyanosis, apnoeic attacks and died at the age of 52 hours.

Cytological Examinations

The chromosomes were studied in lymphocytes from cultures of peripheral venous blood. Numerical analysis of 42 metaphases revealed 47 chromosomes in all the cells. Structural analysis was done in 11 photographic karyotypes. Length measurement and pairing of the chromosomes was easy. The total length of the extra chromosome approximated the length of an 18 chromosome (Fig. 5).

The sex chromatin was studied in amnion and buccal mucosa cells. Out of 100 amnion cells 28 were found to be sex chromatin positive with one Barr body (the study of amnion cells was done because the present case was included in another research programme concerning all the newborn in the "Alexandra" Maternity hospital). Out of 200 buccal mucosa cells 22% were sex-chromatin positive with one Barr body.

Dermatoglyphs

Prints were taken of the palms and fingers using the Walker (11) method but the examination was difficult as the fingers were abnormally flexed.

The most noticeable features were a failure of dermal ridge development on the thenar and hypothenar, abnormal palmar creases on both palms and

This study was supported in part by Research Grants from the National Institute of Neurological Diseases and Blindness of the United States Public Health Service (no. 5R01 NS06390-07) and the Royal Research Foundation (no. 767 and 849).



Fig 1 Patient's head—showing abnormal ears hypoplastic mandible small forehead

simian crease on the right palm. All the finger tips had simple arches.

Pathological Findings

In addition to the macroscopic anomalies previously described, autopsy showed Atrioventricularis communis and patent ductus arteriosus. Hypoplasia of the gall bladder. Ovarian dysgenesis (Fig 6a). Each ovary weighed 0.2 g and the histological picture was that of an undifferentiated gonad. The cortex consisted of undifferentiated deeply stained epithelial-like germ cells which were arranged into partially anastomosed columns. Scattered primordial follicles were found in a few places.

DISCUSSION

The clinical features of the reported case especially those concerning the head and the extremities are characteristic of trisomy 18 as they have been previously described.

Congenital heart disease is very common. It was found in all the cases which had a post-mortem examination (12) except one. The usual type of abnormalities included ventricular sep-

tral defect patent foramen ovale and anomalies of the valves. We did not find any reports of trisomy 18 with atrioventricularis communis, which was found in our case.

Congenital abnormalities of the intestinal tract are frequent, but hypoplasia of the gall bladder is rare. Warkany et al (12) in their review article mention only 2 cases of gall bladder hypoplasia.

Ovarian abnormalities are also rare in trisomy 18 though the ovaries have not been studied thoroughly in all cases which underwent a postmortem examination. The main abnormality was ovarian hypoplasia, the diagnosis being based on the size of the ovaries or on histological examination. Weber et al (13) reported 1 case where the ovaries were small but there were no abnormal histological findings. Butler et al (2) found 2 cases, among seven which were examined histologically, with a



Fig 2 Hand—showing typical position of fingers in the syndrome

marked reduction of the primordial follicles and marked proliferation of the granular cells forming small deeply stained irregular masses. Lafourcade et al (7) reported a case with small ovaries but they did not describe the histological findings. Warkany et al (12) reviewing the findings of 84 autopsies of trisomy 18 found 7 cases of ovarian hypoplasia but they do not say whether the diagnosis was based on the size of the ovaries or on histological findings. Ovarian abnormalities were also reported in 2 cases of trisomy 18 combined with an XXX karyotype (10, 8) and 1 case of XXX plus 18/XXX mosaicism (4). There was great reduction in the number of the primordial follicles with abnormal ovarian stroma. In a fourth case of XXX 18 trisomy the ovaries were found macroscopically to be normal (5).

In our case the ovaries weighed one fourth of the normal weight for the neonatal period and the histological examination showed great



Fig 3 Hand—showing hypoplasia of thenar and hypothenar



Fig 4 Feet—showing short webbed toes and widely spaced big toes

reduction in the number of the primordial follicles. Columns of epithelial like cells were also found. These are considered as granular cells and as elements of the Pflüger columns from which the primordial follicles will be formed later. It is impossible however to say with certainty whether these cellular elements belong to the initially appearing and consequently disappearing cortical columns from which seminiferous tubules are formed in the male (1).

In cases of trisomy 18 with an XXX karyotype the ovarian abnormalities could be ascribed to the extra X chromosome (10) as similar histological abnormalities have often been found in cases with an XXX karyotype without trisomy 18. In our case no abnormality relating to X chromosome was found and therefore we believe that ovarian dysgenesis was an other rare manifestation of the trisomy 18.

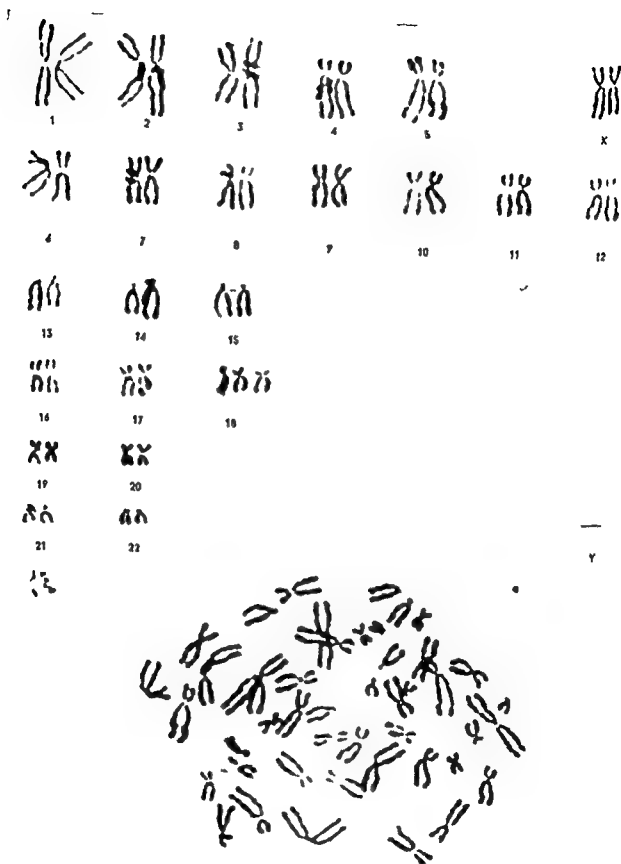


Fig 5 Karyotype of peripheral blood cells culture showing trisomy of chromosome 18 and XX complement (47 XX 18+)

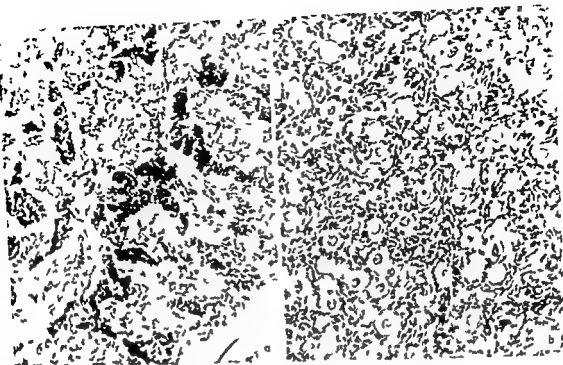


Fig 6 Ovary (a) of the patient showing deeply stained undifferentiated germ cells and (b) of a con

trol case of the same age showing numerous primordial follicles Hematoxylin-eosin stain $\times 120$

SUMMARY

A case of trisomy 18 with ovarian dysgenesis is described. The chromosome study did not reveal any abnormality of the sex chromosomes and therefore we conclude that ovarian dysgenesis is another rare manifestation of trisomy 18.

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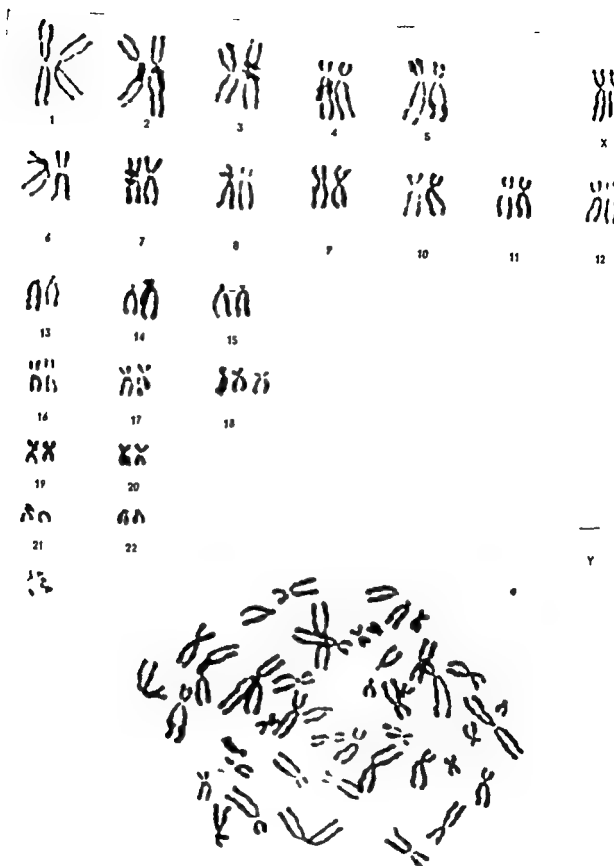


Fig 5 Karyotype of peripheral blood cells culture showing trisomy of chromosome 18 and XX complement (47 XX 18+)

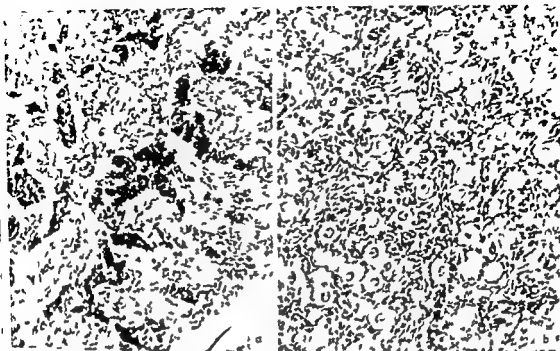


Fig 6 Ovary (a) of the patient showing deeply stained undifferentiated germ cells and (b) of a control case of the same age showing numerous primordial follicles Hematoxylin-eosin stain $\times 120$

SUMMARY

A case of trisomy 18 with ovarian dysgenesis is described. The chromosome study did not reveal any abnormality of the sex chromosomes and therefore we conclude that ovarian dysgenesis is another rare manifestation of trisomy 18.

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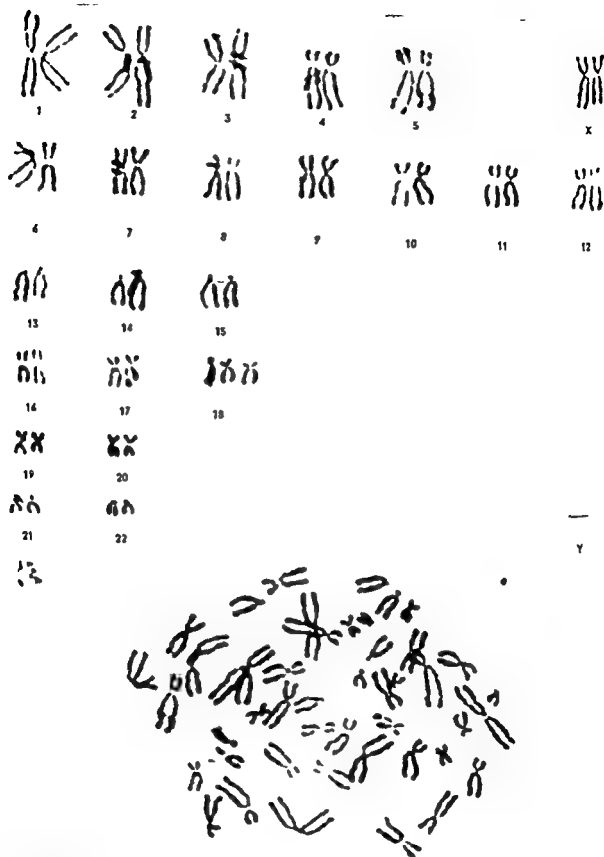


Fig 5 Karyotype of peripheral blood cells culture showing trisomy of chromosome 18 and XX complement (47 XX 18+)



Fig 1 Anne G when aged 3 months

variable abundance. Few inclusions contain other more osmophilic constituents (Figs 2 and 3).

DISCUSSION

There are several reports in the literature concerning infants under 6 months with "gargoylism" (1-4, 8-10). Typical features such as facial dysmorphism, hepatosplenomegaly, joint stiffness, herniae and thickened gums have been observed at birth (1, 6, 10). Bone alterations also reported in the first days of life and characterized mainly by a generalized rarefaction (1, 6, 10) become more typical as soon as 3-6 months (1-4, 6, 8, 9). Corneal clouding was present at 2 and 3 months (3, 4). Mucopolysaccharide excretion normal at birth (6, 10) was noted to be increased after a few weeks (2-4) and vacuolized lymphocytes have been observed at birth. Enzyme and ultrastructural studies are scarcely available in these patients (3, 10).

In our case, several data support the diagnosis of mucopolysaccharidosis type I. Clinically, there exist facial dysmorphism, mental retardation, hepatosplenomegaly, inguinal hernia, joint stiffness and corneal clouding. Biological studies show an increased mucopolysacchariduria affecting both heparan and dermatan sulfate and vacuolized lymphocytes. However, protruding sternum and high dorsal kyphosis are unusual findings. Moreover, the rapidly fatal evolution

is a striking feature which so far has only been reported by Scott et al (10) and Iannaccone & Capotorti (6).

Analysis of acid hydrolases activities in the liver also yields an unusual result. In mucopolysaccharidoses type I to III according to Van Hoof & Hers (12), the activity of β galactosidase is markedly decreased, whereas in our case the activity of this enzyme remains within normal limits. This latter fact may not be related to the early age of the infant since in Corbeel's patient the activity of β galactosidase was markedly decreased.

Finally, the ultrastructural aspect is not entirely similar to that observed in mucopolysaccharidoses type I to III. In this group of diseases, the different liver cell types contain large clear inclusions (3, 5, 7, 11). In the present case, the inclusions are similar in content but they are much more numerous and generally smaller. They often display evidence of coalescence, possibly leading to the formation of larger vacuoles.

SUMMARY

A mucopolysaccharidosis is diagnosed in an infant aged 3 months. Clinical and X-ray data are not typical, while biological studies reveal an increased mucopolysacchariduria affecting both heparan and dermatan sulfate. Study of lysosomal hydrolases in the liver discloses a normal activity of β galactosidase. Hepatic ultrastructure

CASE REPORT

MUCOPOLYSACCHARIDOSIS IN A THREE MONTHS OLD INFANT

Clinical and Ultrastructural Studies

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CASE REPORT

Anne G ■ female infant was born on February 14 1968 after 8 months gestation She is the first child of a single woman aged 19 the father is unknown Delivery through breech presentation was uneventful Birth weight was 2 450 g Postnatal clinical examination only reveals a very large fontanel Hypotony and hyporeactivity are noted during the first 2 months of life and thoracic difformity at the seventh week When 2 months old the child is hospitalized for the first time for respiratory distress with cardiac failure Her height is at the 50th percentile (57 cm) whereas her weight is below the 10th percentile (3 490 g) Head circumference is at the 90th percentile (39.5 cm) and the fontanel is abnormally large (5 × 3 cm) Bulging forehead flattened nose with depressed nasal bridge as well as prognathism of the superior jaw give to the facies an obviously dysmorphic aspect (Fig 1) Palate is high arched There is a very important dorsal superior kyphoscoliosis The thorax whose antero posterior diameter is markedly increased is deformed by the broad precordial arching There is no cardiac murmur A hepatosplenomegaly persisting after correction of the cardiac failure is noted Other anomalies include a moderate but generalized limitation of joint movements with thumbs kept in palmar flexion as well as a left inguinal hernia

X ray studies disclose a deformed thorax with a high dorsal kyphosis and a markedly protruding sternum Vertebral bodies show the oval aspect usual at this age Tubular bones do not reveal any alteration Electrocardiogram is normal Caryotype and chromatography of urinary aminoacids yield no abnormality Toxoplasmosis and rubella antibodies are absent Fundi and corneae are normal In peripheral blood 8% of the lymphocytes display abnormal vacuoles containing dense central granules Bone marrow is normal Urinary excretion of mucopolysaccharides expressed in mg hexuronic acid is increased (21.5 mg

per liter) Column chromatography performed according to a previously described technique (5) isolates important fractions corresponding respectively to heparan and dermatan sulfate Fragments of liver are obtained through puncture biopsy and submitted to enzyme and ultrastructural studies

When 31 months old the child is discharged but rehospitalized 3 weeks later because of another episode of respiratory distress Height and weight curves show a marked inflexion Hepatosplenomegaly is more conspicuous Slit lamp examination of corneae reveals the presence of fine granular opacities Psychomotor development is considerably delayed and corresponds to an age of 1 month This delay affects more postural control than social reactions The child leaves the hospital when 5 months old and dies at home 1 month later after a third episode of acute respiratory distress

Enzyme studies

Activities of liver acid hydrolases were assayed by Van Hoof and Hers in the Laboratory of Physiological Chemistry of the University of Louvain The activity of β galactosidase is more than four times higher than the mean activity observed by these authors in a group of 14 patients belonging to the mucopolysaccharidoses type I to III (12)

Electron microscopy

In most hepatocytes and Kupffer cells, the cytoplasm is filled with large clear inclusions limited by a unit membrane Their main component is a moderately osmophilic substance scattered as a fine protein like precipitate of

ture shows in both parenchymal and Kupffer cells the presence of clear inclusions analogous to those found in typical mucopolysaccharidoses although more numerous and generally smaller

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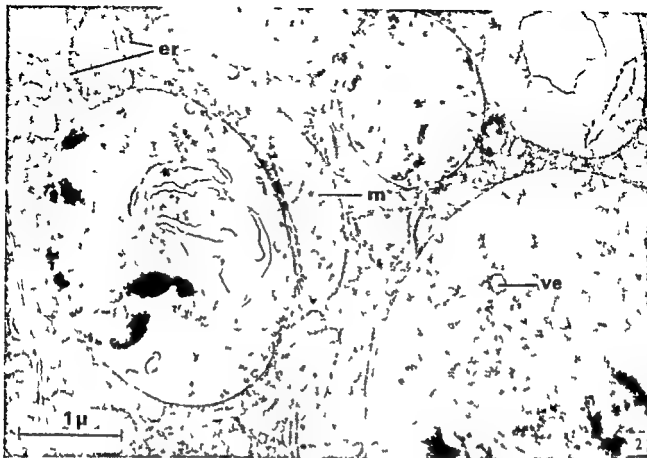


Fig 2 Hepatocyte. Large clear inclusions contain a moderately osmophilic protein like precipitate coalescing sometimes in more dense areas i.e. small vesicles limited by a unit membrane *m* mitochondria *er* ergastoplasm $\times 22\ 000$

Fig 3 Hepatocyte filled with smaller inclusions. Some of them contain a few myelin like elements (*my*) and/or osmophilic granules (*arrows*). Coalescing figures of several inclusions are observed *g* a glycogen *en* dilated endoplasmic reticulum $\times 22\ 000$

Another infant who was in a respirator for a 24 hour period with an oxygen concentration of as much as 90% later developed a subdural hygroma which was excised. Evidence is now present of delayed psychomotor development and non specific abnormality on the EEG.

The third infant was delivered in the 33rd week of gestation and showed severe asphyxia. During the first weeks of life this patient developed pneumothorax in conjunction with respirator treatment and had recurrent episodes of cardiac arrest. Respirator treatment extended over a period of 4 months and the patient was extubated at 7 months. There is evidence of significant respiratory involvement with tracheomalacia, marked retractions and hyperinflation on roentgenograms of the chest. The EEG shows non specific abnormality with left sided temporal preponderance. However the psychic and psychomotoric development is normal.

Bengt Hagberg & Leif Hambræus Atypical case of leucinosia (maple syrup urine disease)

A 9 year-old boy with a clinical picture of dwarfism, microcephaly with imbecility, severe motor retardation and congenital cataracts is described. The patient was born 3 weeks before term and his birth weight was 1 860 g. During the neonatal period he had a clinical history consistent with a prenatal brain damage and was in a bad condition with convulsions and abnormal neonatal neurology. At repeated follow up examinations it was found that the boy's somatic and psychomotor development was markedly delayed.

At repeated occasions this boy had very peculiar and unexplained episodes lasting 6-12 hours and consisting of vomiting, a semicomatous state and sometimes also convulsions.

During one of his episodes a biochemical study of the amino acid pattern in blood, cerebrospinal fluid and urine revealed a marked increase in the concentrations of the branched

amino acids valine, leucine and isoleucine. This pattern was most pronounced in the blood and cerebrospinal fluid. In the urine abnormal concentration of ketoacids was demonstrated. When the patient's episodes had stopped these abnormalities were normalized. It is suggested that this patient represents a transient and atypical form of leucinosia (maple syrup urine disease).

Bengt Hagberg, Hans Kollberg, Patrick Sourander & Hans Olof Akesson Infantile globoid cell leucodystrophy (Krabbe's disease)

A series of 32 cases of globoid cell leucodystrophy was collected from a 15 year period and studied particularly from clinical and genetic aspects. The series was considered representative of the real occurrence of the disease in Sweden during the period in question.

The clinical picture and the course of the disease were usually surprisingly uniform from case to case. The median age at onset was 4 months and at death 13 months. The course of the disease was divided into three clinical stages the characteristics of which have been described in detail.

Laboratory aids for diagnosis have been discussed on the basis of experiences from this series. Histological changes for diagnosis were summarized from studies of biopsy specimens from peripheral nerves and brain.

A geneto-statistical study showed that the probands with GILD did not differ from the standard population in either maternal age or birth rank. That there was no apparent consanguinity in this series, that the estimated incidence was approximately 1.9×10^{-6} and that the disease was diffusely spread over the country. Finally from the family analysis it was concluded that globoid cell leucodystrophy is most likely inherited as an autosomal recessive disease.

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC SOCIETY

Meeting April 26, 1969

Lars Victorin, Anders Gustafson Ingemar Kjellmer & Ragnar Olegard *Intravenous administration of fat to premature infants*

Early administration of intravenous fluids and calories appears to reduce both the morbidity and mortality of prematurely born infants. Administration of a 10% solution of invertose (65 ml/kg daily) is commonly used but probably only amounts to one third or one fifth of the actual requirement. As a mean of increasing the caloric intake intravenous fat in the form of Intralipid Vitrum (0.5 g/kg in a single dose) was administered during a 5 min period. This solution provides about 2 cal/ml, which is roughly four times more per volume unit than the above-mentioned invertose solution.

This intravenous fat solution may be equivalent to endogenous chylomicrons. It is metabolized in the same manner as orally administered fat apart from the fact that it lacks a protein which must be supplied by plasma if separation of triglycerides is going to occur.

In 10 cases intravenously administered fat was rapidly metabolized and the initial hyperlipemia was in most cases no longer evident after 2 hours and had a half life of 20-30 min. In a few cases the level of total lipids remained elevated for a longer period—between 6 and 30 hours. These cases were further analyzed by paper electrophoresis and agarose electrophoresis. It could be shown that this was not due to delayed handling of the chylomicron fraction in the liver. The level of this fraction falls at the same rate as that seen in the other

prematures. The relatively high total lipid level between 2 and 30 hours of age was due to pre-beta lipoproteins—in other words second generation lipoproteins formed in the liver.

In all cases the blood sugar concentration was above the hypoglycemic limit (30 mg/100 ml). No pathological increase in ketone bodies was observed.

Lars Victorin & Roy Cooke *Follow up of infants with the idiopathic respiratory distress syndrome (IRDS)*

During a period of eighteen months dating from January 1967 until June 1968 all surviving infants with the diagnosis of IRDS (hyaline membrane disease) have been re-examined. They were between 8 and 24 months of age at the time of this study. The severity of their illness was classified as mild in 4 cases, moderate in 15 and severe in 7. Five of these 7 patients were treated in a respirator. The examination included careful physical and neurological evaluation, ophthalmological and audiological testing, roentgenogram of the chest, EEG as well as developmental evaluation at home using Lindstrom's (1967) modification of Griffith's developmental scale. No definite pathological finding was noted in 23 of the 26 cases.

One patient who required respiratory treatment until the age of 6 weeks showed mild retractions with stridor. In addition the roentgenogram of the lungs showed alternate regions of hyper- and hypo-inflation.

they are usually short and blind. From a roentgenological point of view it can then sometimes be impossible to distinguish between ulcus and fistulae.

The roentgenographic appearance of local foci with submucosal hyperplasia resembles that of polyposis and is the basis of another type of cobble stone pattern which is less coarse than that referred to above.

Segmental involvement with alternating healthy and diseased segments of the intestines is common. Sometimes it is only the mesenteric side of an intestinal segment that is affected and spastic. In such cases the opposite healthy side will bulge like a diverticulum.

The roentgenogram of granulomatous enterocolitis contains a number of inflammatory changes that are not characteristic of this disease alone. But the combination and the site of the changes are usually characteristic enough to allow differentiation of the condition from other diseases.

N O Berg, Pathology

In 1966 Ehrenpreis et al. discussed 17 juvenile subjects with Crohn's disease. In 13 of these surgical resection of diseased intestinal segments was performed. Of our 13 subjects only one was operated upon. In this the ileocaecal region presented the classical morphologic lesions with tuberculoid granulomas in the mucosa and in the enlarged lymph nodes.

Peroral suction biopsies of small and large intestine and rectal biopsies showed intestinal lesions in another 9 subjects. Of 38 specimens all sectioned serially in its entirety no abnormality was seen in 19. Multiple biopsies (3-9) were performed in 6 subjects and single biopsies in three.

Characteristic lesions such as tuberculoid granulomas were found in three subjects. Brunnerian gland metaplasia, lymphoedema or single giant cells were seen in a few biopsies. In six specimens from three different subjects a flat villous mucosa was seen in roentgenologically changed intestinal segments. The changes dif-

fered from those seen in coeliac disease in mainly two respects: the changes were more granulomatous with more neutrophils and they reached down to the submucosa. Of special interest was the occurrence in four subjects of small focal lesions in intestinal segments without distinct roentgenological changes, mostly in the upper part of jejunum. The diagnostic and pathogenetic significance of these discrete lesions is not settled.

U Cavell, Gastric emptying in infants

A new method for studying gastric emptying of human milk in infants is described. The method makes it possible to study the whole emptying pattern of one meal without the need of complete aspirations and refillings of the stomach. The technique involves gastric intubation and iterated intragastric volume determinations using polyethylene-glycol (PEG) as a marker.

Known amounts of concentrated PEG solution are given via the tube at each volume determination and the resulting increase in PEG concentration of the gastric contents after mixing is measured. Knowing this concentration the intragastric volume can be calculated.

The gastric emptying pattern in infants aged 2-6 weeks showed an initial rapid emptying phase followed by a slower phase with a constant emptying rate of 0.4 ml per minute and lasting until the stomach was empty. In some infants aged 2-3 months however an exponential emptying pattern with a constant of decrement about 1.5-2.5% was found.

The variations of gastric emptying pattern from day to day within individuals seemed to be rather small.

N W Svenningsen, A Dahlqvist & B Lindqvist, *HBABA index in neonatal icterus*

Introduction

B Lindqvist. The relationship between the serum bilirubin concentration and bilirubin en-

Meeting Oct 10, 1969

REGIONAL ENTEROCOLITIS
(CROHN'S DISEASE)B Hansing & G Meeuwisse *Clinical signs*

From 1963 until October 1969 we have seen 13 new cases of regional enterocolitis at the Paediatric Department, University of Lund. The age distribution was 6-14 years with maximum 4 cases at 10 years. There were 3 girls and 10 boys. One of the patients has an elder sister with the disease. During the same time we had 17 cases of ulcerative colitis (14 girls and 3 boys). The diagnosis has been established on the clinical picture in combination with distinguishing radiological features, in some cases supported by pathological studies on intestinal biopsies. The primary symptoms were diarrheas (4 cases), abdominal pain (5), suspect appendicitis (1), erythema nodosum (1), anal fissure (1), elevated ESR and gammaglobulin (1). Moreover, we observed digital clubbing (2 cases), short stature (2), delayed puberty (1), oedema (1), loss of weight (13), fever (6). All patients were anaemic with sideropenia and in most cases low TIBC. 6/8 had subnormal levels of folic acid/s. 8/8 had normal B₁₂/s. 12/13 had elevated ESR. Albumin/s was generally low. Gammaglobulin/s was 2.10 in one case and above 1.20 g/100 ml in 6 other patients. 3/3 had elevated ASTA. 3/11 had a pathological xylostep. RISA test showed in 2/2 cases proteinlosses in faeces more than 30 times the normal.

In 3 cases there had been difficulties before establishing the correct diagnosis. One of these had at another hospital passed a thorough endocrinological examination because of short stature, another was retarded in his growth, had oedema but normal ESR, the third had elevated ESR and gammaglobulin but no symptoms directly referable to intestinal disease.

All patients were treated with salazopyrin. One had a stop medication after 3 weeks because of exanthema. Another got his treatment only for one week and was then referred to the surgical department. The rest have been

treated more or less continuously even during symptomfree periods. The only surgically treated patient has just relapsed, the other patients are now in a good general condition. In 5 cases we have added steroids in general low doses during short periods. Especially in the 2 cases with proteinloosing enteropathy we have seen a very good response to this medication. Salazopyrin alone did not improve these patients. In 2 cases with intermittent salazopyrin treatment we could see a prompt effect of salazopyrin with rapid increase in weight and cessation of symptoms.

W Mortensson *X ray picture*

Inflammatory lesions of the intestinal wall and mesentery produce a widely varying picture as may be seen in roentgenograms of patients with granulomatous enterocolitis. The lesions are situated mainly in the small intestine. They are of essentially the same appearance in the large and in the small intestine. The thickening of the intestinal wall causes narrowings of the lumen and the valvulae conniventes become irregular, blunt and ill-defined. Spasms due to the inflammatory changes also affect the width of the lumen in the acute stage of the disease. In later stages cicatrization may cause permanent strictures. Intestinal dilatation is rare, but may occur orally to the stenosed segment.

Inflammatory thickening of the mesentery and of the wall of the small intestine deforms the otherwise smooth curves of the loops; the loops are separated from one another and assume a rigid course.

The ulcerations usually extend longitudinally in the mesenteric side of the intestine and are often connected with one another by transverse ulcers with the formation of a cobble stone pattern. The ulcerations may also be deep and perpendicular of diffuse and simulate the absence of mucosa.

Fistulae may communicate with other hollow organs or with the surface of the body. But

they are usually short and blind. From a roentgenological point of view it can then sometimes be impossible to distinguish between ulcers and fistulae.

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N. W. Svenningsen, A. Dahlqvist & H. Lindqvist HBABA index in neonatal icterus

Introduction

B. Lindqvist The relationship between the serum bilirubin concentration and bilirubin en-

cephalopathy has been well established in Rh-hemolytic disease. In nonhemolytic hyperbilirubinemia this relationship is, however, less well defined. This is especially relevant for premature infants. It is now well known that there are many factors contributing to the pathogenesis of bilirubin encephalopathy: rate of hemolysis, development of the enzyme systems, acid-base balance, presence of hypoxia and respiratory distress, administration of certain drugs, etc. Since 1959 Odell and co-workers have in several reports pointed out that the relationship between the concentration of serum albumin and serum bilirubin is a better criterion than the bilirubin concentration alone in judging the risk for bilirubin encephalopathy. This concept is based on experimental studies with ^{14}C -bilirubin; the concentration of ^{14}C -bilirubin in the central nervous system was correlated to the amount of free bilirubin (bilirubin not bound to albumin) rather than to the total bilirubin level in serum. Bilirubin encephalopathy will arise when the serum albumin has an insufficient amount of binding sites for bilirubin.

By *in vitro* studies the amount of bilirubin that can be bound to a certain amount of albumin can be determined and thus the relative amounts of free and bound bilirubin in a mixture of bilirubin and albumin can be calculated. The situation is, however, more complicated *in vivo*: the binding capacity varies, a due to presence of exogenous and endogenous substances which compete with bilirubin for the binding sites of the serum albumin. Therefore it has been necessary to work out special methods to determine the so called reserve albumin (the reserve capacity of serum albumin to bind unconjugated bilirubin). Knowledge of the amount of reserve albumin in the single patient will probably give a more accurate background for evaluation of the risk for bilirubin encephalopathy than does information about the bilirubin level alone.

Method

A. Dahlqvist The measurement of reserve albumin is based on the ability of albumin to

bind a suitable dye indicator. This dye must be bound to albumin with a lower affinity than is the bilirubin, else the dye will replace bilirubin and the total albumin rather than the free (reserve) albumin will be measured. A method utilizing PSP (phenolsulfonphthalein) was described by Waters & Porter in 1961.

This method had the drawbacks of being laborious and time consuming and the amount of serum needed is fairly large. A better method was described by Porter & Waters in 1966, utilizing HBABA (2-(4-hydroxybenzeneazo) benzoic acid) and this is the method we have used. The colour of the HBABA albumin complex is different from that of free HBABA, and therefore, if the measurements are made at suitable wavelength, the amount of HBABA albumin complex can be measured without separating it from the free dye. This method is rapid (it is performed in a few minutes) and the amount of serum needed is small (0.2 ml, including the amount used for blank correction). In our experience it is accurate and reproducible. The standard deviation was $\pm 2.9\%$ of the mean value when calculated from 30 assays of the same sample (each assay performed on a separate day).

The results of HBABA-binding assay are expressed as an index value where index 100 refers to a standard solution containing 4 g albumin per 100 ml. The normal range is an index value of 70-100, and a value below 25-30 is supposed to indicate a risk for the development of bilirubin encephalopathy. In a number of samples from patients with different degrees of icterus we compared the experimentally obtained value for the HBABA index with a theoretical value calculated from the assay of total albumin and bilirubin respectively. The albumin concentration was determined by quantitative electrophoresis. In a few cases the assay of albumin was also performed with high voltage immunoelectrophoresis (these assays were performed by C. B. Laurell, Malmö). The theoretical HBABA index was calculated with the assumption that 1 gram of albumin can bind 17 mg of bilirubin which is the relation

Table 1 *Infants with bilirubin encephalopathy—clinical signs or verified kernicterus at autopsy (ki)—at HBABA index levels above or below 30 in group II*

HBABA index	Total no	Neonatal deaths		Surviving infants with bilirubin encephalopathy	
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> 30	138	11	1	1	—
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found in test tube experiments. The experimentally obtained HBABA index was in these sera up to 60% lower than the theoretical HBABA index. Only in a few cases did both methods give the same index figure. This is in good agreement with the concept that in addition to bilirubin some other substances (endogenous and exogenous) are bound to albumin and that therefore the dye binding capacity of the serum should be a better measurement of the amount of reserve albumin available than are separate measurements of bilirubin and total albumin.

Clinical investigation

A. B. Sienningsen. The clinical investigation comprises 3 groups of neonates 0 to 7 days of age. **Group I** 46 infants—22 preterm and low birth weight infants (LBW) and 24 fullterm infants (FT)—with a maximal serum bilirubin of 6.2 mg/100 ml during the neonatal period. A significantly lower HBABA index on days 1 to 4 was found among the nonicteric LBW infants.

Group II 175 infants—86 LBW—and 89 FT infants—with hemolytic (50) and nonhemolytic (125) hyperbilirubinemia. Serum bilirubin and HBABA index were followed concomitantly in the neonatal period. A significantly lower HBABA index among the LBW infants was demonstrated even in this group.

A higher incidence of bilirubin encephalopathy was demonstrated among infants with low HBABA index (group II) irrespectively of the serum bilirubin level (Table 1). Eight out

of the 11 (4 + 5 + 2) infants with low HBABA index and symptoms of bilirubin encephalopathy or kernicterus verified at autopsy were LBW infants. Maximal serum bilirubin values between 12.0 and 17.1 mg/100 ml were registered in the cases with kernicterus. In comparison to this no kernicterus was found at autopsy of another 9 infants with the same serum bilirubin level, the same gestational age and birth weight but with HBABA index in the normal range above 30. The indications for exchange transfusion in this group were the empirically accepted serum bilirubin levels considered at risk.

Group III 99 infants—46 LBW—and 53 FT infants—with hemolytic (28) and nonhemolytic (71) hyperbilirubinemia. These infants were treated irrespectively of the serum bilirubin level with albumin infusion and exchange transfusion when HBABA index decreased rapidly towards or below 30. No kernicterus has been found among 7 infants dying in the neonatal period although HBABA index was below 30 in 3 infants. Neither have any late cerebral sequelae been registered among the survivors. The mean follow up period is now 11 months. When albumin infusion (1 g/kg/day) was given a mean rise of serum bilirubin of 3 to 5 mg/100 ml was found within 6 to 8 hours. This is probably the result of a bilirubin shift from extra to intravascular compartment.

In a separate series of 52 infants with non hemolytic hyperbilirubinemia the effect of albumin enrichment of donor blood (50 ml 20% human albumin per 350 ml donor blood) was studied. Albumin enrichment was performed

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In a separate series of 52 infants with non-hemolytic hyperbilirubinemia the effect of albumin enrichment of donor blood (50 ml 20 human albumin per 350 ml donor blood) was studied. Albumin enrichment was performed

at every second exchange transfusion in this series. A significantly higher and persisting rise of the HBABA-index was demonstrated in the albumin enriched cases.

In summary the results of this investigation have demonstrated that the HBABA-index is a most useful and reliable test for evaluating the recombining capacity of the serum albumin. A good correlation between low HBABA-index and the incidence of bilirubin encephalopathy was found even at serum bilirubin levels below the empirical levels for exchange transfusion. Albumin infusion resulted in a significant rise of HBABA index. As a consequence of these findings it is considered that exchange transfusion should be performed in some infants with relatively low serum bilirubin level, but at the same time also low HBABA index. On the other hand exchange transfusion should not necessarily be performed in other infants with serum bilirubin above the conventional levels for exchange transfusion if the HBABA-index level is high.

G Engleson, S Aronson & J Thorell *The Prader test and plasma HGH in children with short stature*

Short time metabolic test with exogenous human growth hormone, HGH, the Prader test has been used by us as a diagnostic measure in 4 patients (*Arch Dis Child* 39 535, 1964). The test was performed as a balance study. The protein and caloric intake of the patient was fixed. The nitrogen excretion in the urine was estimated from Urea-N + Ammonium N. The patient was given HGH (by courtesy received from M. S. Raben) and the percentual decrease of the urinary N was computed. The plasma levels of HGH at the intravenous insulin test and arginine load were compared with the findings of the Prader tests. Two patients had maximal values of plasma HGH less than 6 ng/ml. These children retained pathological amounts of nitrogen, 43 and 44% respectively

at the Prader test. One patient who had normal plasma HGH values showed no N retention. The fourth child had intermediate values in plasma HGH. Her maximal value at two insulin tests was 11 ng/ml. Her N retention was slightly elevated, 21%.

The diagnostic value of the Prader test has been questioned. So far our data show a good correspondence between the results of this test and plasma HGH values.

Lately another child with short stature has been found to have plasma HGH less than 6 ng/ml. He also had an increased N retention of 36% at the Prader test.

N-R Lundström *Ultrasoundcardiography in infancy and childhood*

Ultrasoundcardiography has been performed on more than 200 children. The age of the children has varied between 1 day and 16 years. Most of the investigations have been performed without premedication and all without discomfort for the patient.

The method has been found valuable for demonstration of dilatation of the right ventricle both in cyanotic and acyanotic congenital heart disease. In the study of the tricuspid valve we have found pathologic findings in tricuspid atresia and Ebstein's anomaly.

The method has been found valuable in evaluation of the state of the mitral valve. Pathological findings have been obtained in congenital mitral stenosis, mitral atresia and other forms of hypoplastic left heart syndrome, cleft mitral leaflet in endocardial cushion defects and other forms of mitral regurgitation.

We have also studied the outflow tract of the left ventricle. Pathological findings have been obtained in cases with membranous subvalvular aortic stenosis and hypertrophic obstructive cardiomyopathy.

Even in infants and children the method has been useful for demonstration of pericardial effusion.

Hans Ahlstrom *External detection of ^{51}Cr Hippuran in the diagnosis of left to right shunts*

Cardiac left to-right shunts can be discovered by external detection over the lung field after intravenous injection of a radioactive isotope. In 35 patients (adults and children) ^{51}Cr Hippuran was injected during cardiac catheterization and a scintillation detector was placed over an apical lung field. The peak activity (C_2) and the activity after an interval equivalent to the build up time (C_1) were measured. Oxygen saturation data were utilized for quantitation of the magnitude of the shunts. These results were compared with the ratio of activity C_2 to C_1 expressed as a percentage.

In 15 patients without left to-right shunts C_2/C_1 were less than 50%. In 5 patients with small shunts (lung flow/systemic flow less than 2/1) C_2/C_1 were 50-65%. In patients with large shunts (2/1 or more) the ratio were more than 65%.

The method is simple and should be valuable in children and it seems to be a reliable method in screening patients with heart murmurs of uncertain etiology.

R Stensman & B Ursing *Epilepsia termobal nebris—an unusual case of reflex epilepsy*

A case of reflex epilepsy in an otherwise healthy 5 year-old boy was presented and briefly discussed. When the patient was taking a hot bath he had attacks of focal temporal type and simultaneously left temporal abnormality appeared in the otherwise normal EEG. The precipitating stimulus was found to be specific. Water with low temperature, hot air, among other tests did not provoke seizures or EEG abnormalities. Antiepileptic treatment with carbamazepine (Tegretol®) has so far been successful.

Lars Holmberg *Solitary mastocytomas*

Solitary mastocytoma is a form of mastocytosis with only one isolated skin lesion. Eight cases

of solitary mastocytoma among children born in 1966-68 in Malmö have been recorded. Six of them have been submitted to close investigation. In most cases the tumour appeared at birth or soon after. The diagnosis was confirmed by histological examination. No signs of systemic mastocytosis were revealed by X-ray survey of the skeleton or by bone marrow studies.

One patient had an attack of generalized flushing and another had an attack of bronchial asthma on mechanical stimulation of the tumour. In the first case there was an increased urinary excretion of histamine while it was normal in all the others. The urinary excretion of 5 HIAA was normal. The coagulation mechanism was normal and in particular no pathological amounts of a circulating anticoagulant of the heparin type were demonstrated. Furthermore there was no local fibrinolytic activity in the tumour itself.

Inger Magnusson & Sven Åke Kornfalt *Wilms's tumour*

The material was collected from the Departments of Radiotherapy and Paediatric Surgery, University Hospital, Lund. It covers a 15 year period (1953-1968).

From 1958 to 1963 the number of new cases diagnosed each year in Sweden varied between 11 and 19.

The present material consisted of 33 children (15 boys and 18 girls). The tumour was bilateral in 5, left sided in 17 and right sided in 11.

One patient received no treatment. The remaining 32 were distributed among three groups according to treatment.

Group 1 (3 patients). Only excision of the tumour. No survivors.

Group 2 (6 patients). Preoperative radiotherapy followed by excision of the tumour and postoperative radiotherapy. One survivor.

Group 3 (23 patients). Immediate nephrectomy followed by postoperative radiotherapy.

Nine survivors Of the survivors, 6 were followed up for more than 5 years 1 for 5 years, 1 for 2 years and 1 for less than 2 years Five of the 9 survivors received orthovoltage X-ray (170 kV, 1.0 mm Cu HVL) and the remaining four ^{60}Co irradiation

The initial symptoms were abdominal swelling or a palpable mass in 26 cases, pain in 12, nausea and vomiting in 9 and hematuria in 6

Of the 32 patients treated 10 have survived and nine of them were followed up for more than 2 years and showed no signs of recurrence when last seen Eight of them belonged to group 3 (nephrectomy followed by postoperative radiotherapy) Immediate nephrectomy plus simultaneous administration of Actinomycin D followed by postoperative radiation is recommended

The importance of early diagnosis and centralization of treatment and close cooperation between the paediatric surgeon and the radiotherapist is stressed

II Lindquist & G Meeuwisse *Should gastroenteritis in infancy be treated with antibiotics?*

Starting with a case history of a child who—because of an ordinary enteritis—within a few days was given different antibiotics but no dietary prescription, the drawbacks of routine treatment of gastroenteritis with antibiotics were discussed Some recently published articles propagating the routine usage of Neomycin in children below 2 years of age hospitalized for gastroenteritis demonstrate that the disadvantages of such and similar practice are not always given due consideration Slowly absorbable antibiotics like Neomycin may cause parenteral and enteral side-effects Parenteral side effects are the consequence of absorption to an unforeseen degree Risk of an enhanced absorption exists in the neonatal period but also later on in life if the intestinal tract is subjected to inflammatory changes By this mechanism Neomycin may cause deafness and renal damage The enteral side effects consist

of 1) disturbed intestinal micro-flora, including the risk of dominating growth of e.g. *Staphylococcus aureus* or *Candida albicans* 2) increased transfer of resistancy factors between strains and sorts of bacteriae In this way bacteriae (e.g. *Proteus*) with multiple resistancy will arise and they will constitute a potential risk for the environment of the patients As in countries like Sweden acute gastro-enteritis is rarely caused by bacteriae the use of antibiotics is not indicated unless we are dealing with a documented epidemic with a pathogenetic *E. coli* strain Moreover, routine administration of antibiotics may lead to diminished interest for dietary treatment and ignorance of instructions for barrier ward which are issued to prevent spread of diarrhoeic disorders within the hospital

Jan Lagergren & Richard Stensman *EEG and the clinical course after cerebral hypoxia in childhood*

An almost 3 year-old boy was deeply unconscious after an accident for about 24 hours and regained consciousness gradually Two weeks after the accident he could sit up without support and started to talk again After 4 weeks he could walk unsupported In spite of the good and continuous clinical improvement there was in the EEGs an increase slowing with maximum on the 17th day after the anoxia It was not until two months after the accident that the EEG was normal

The other boy was 12 years old when he by accident was strangulated He was unconscious for about 15 hours From the second day after the accident he was active concentrated and physical examinations showed normal motor and neurological findings An initial EEG showed an increased amount of slow activity bilaterally After a slight improvement there was—in spite of the clinically excellent condition of the patient—a deterioration of the EEG with a maximum on the 6th and 8th day The

increased amount of slow activity was then decreased again and the EEG was quite normal one month after the accident

In our 2 cases the discrepancy between the clinical improvement and the late changes in the EEGs is interesting. Delayed neurological deterioration following anoxia has attracted little attention in the literature. In 2 patients

dying from brain damage after anoxia an extensive demyelination was found. The background to the late EEG changes in our cases may be of similar nature.

We think more intense investigations with serial EEGs are of great interest in cases with cerebral hypoxia and necessary for evaluation of the prognosis.

R. Lagercrantz,

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting Febr 14 1970

J R Bierich (Tubingen West Deutschland)
Etiopathology and modern treatment of sexual precocity

Meeting Apr 24, 1970

Katri Malmivaara *Psychiatric consultations in paediatric hospital wards*

Experience derived from psychiatric consultations on paediatric wards at Aurora Hospital, a general city hospital in Helsinki, was presented. There has been a small psychiatric unit at this hospital for 18 years. Regular psychiatric consultations have been available for paediatric patients since 1957. These include case conferences together with paediatricians, psychiatric examinations, different kinds of therapy (individual psychotherapy for children and parents, group psychotherapy, family therapy) and supervision of medical staff on children's wards. An analysis of the obstacles and achievements in this work was presented.

when a paediatrician finds out that the patient needs psychotherapy and thus disrupts his or her ordinary time schedule. Even if it would be possible to increase the amount of training in psychology, sociology and childpsychiatry, a keen interest in the developing personality of a growing child is required in order to become a successful psychotherapist.

Juhani Lappalainen

Gunvor Vuoristo *Brief Psychotherapy*

For a paediatrician in daily practice it is possible to prevent neurosis, if he is interested in psychotherapy. When child psychiatric consultation is available there may still be difficulties in communication due to differences in the quality of language of a paediatrician and a child psychiatrist. Another difficulty turns up

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SECOND INTERNATIONAL SYMPOSIUM OF PAEDIATRIC NEPHROLOGY

The 2nd International Symposium of Paediatric Nephrology will be held in Paris from 25-28 August 1971

President P Royer

General Secretaries R Habib and H Mathieu

The scientific programme will deal with the following topics (1) Developmental nephrology (2) Immunological aspects of Paediatric Nephrology (3)

Chronic uremia and its treatment (4) Free communications

The languages of the symposium are English, French and Spanish. Attendance will be limited to 350 participants. Active investigators in the field interested by the Symposium are requested to inform as soon as possible Dr Renee Habib, Hospital des Enfants Malades, 149 rue de Sevres, Paris 15, France.

ASSOCIATION FOR PAEDIATRIC EDUCATION IN EUROPE

After various surveys, discussions and seminars on paediatric education in Europe sponsored by the European Office of the World Health Organization, it was felt that the time has come for the foundation of an official Association. The main purpose of which will be the paediatric education at all levels in all countries of the European region. Numerous informal meetings and discussions took place until on September 29th 1970 in Athens the new Association was officially founded. It is called Association for Paediatric Education in Europe (A.P.E.E.) and the aims are:

- a) To encourage improvements in paediatric education

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The first Executive Committee was elected consisting of the following: G.M.H. Veeneklaas, Leiden; President S.A. Douzidis, Athens; Secretary Treasurer J. Houstek, Prague; Nathalie Masse, Paris; S. Solin, Uppsala.

The next meeting of the Association will take place in Vienna before the 13th International Congress of Paediatrics. The address of the Secretary is: Institute of Child Health, Athens 603, Greece.

EUROPEAN STUDY GROUP ON CHILD NEUROLOGY

Under the sponsorship of the Spastics Society of England and arranged by its Medical and Educational Unit directed by Dr R. Mac Keith, a meeting of 57 specialists in child neurology was held in Oxford during the last week of September 1970 for discussions on future international communication. It was considered that much could be gained by following the pattern of meetings which have taken place in Oxford for more than 17 years by courtesy of the Spastics Society of England. The value of such meetings has lain in the fact that they have been limited to 50-60 participants who have given papers and have profited from free and extensive discussions. It was decided therefore to organize a "European Study Group on Child Neurology". This would convene every third year and would comprise a permanent body of 30-40 members and in addition partici-

pants selected for each coming meeting by a council according to the subjects to be discussed. Such participants might very well be experts from other fields than pure clinical child neurology. The total number of persons taking part would be limited to 60.

A preliminary council of 6 members was elected representing different regions of Europe: namely Dr Neil Gordon (United Kingdom and Ireland); Dr Gilles Lyon (France/Italy/The Peninsula); Dr Jan Willemse (Benelux); Dr Franz Schulte (Central Europe); Dr Ivan Lesny (East Europe); and Dr Benot Hagberg (Scandinavia). The council elected Dr Hagberg as its secretary and Dr Sven Brandt was appointed chairman of the first meeting to be held in Scandinavia in the autumn of 1973.

Sven Brandt

aspects of the progressive brain disorders would have been quite an advantage

The book presents itself as a nice printing but it is unavoidable that there are printing errors hope fully only few. Many of the figures are of high quality

The part on psychology and psychiatry offers much interesting reading and includes some remarks on children in hospital, child-doctors relation etc. which is rare and valuable to find in such a work

This book is of value to a complete library both for readers with a special interest in neuropaediatrics and related areas as well as for persons interested in infections of the CNS and many other problems

Personally I believe that in order to keep the reader up-to-date smaller books on single parts of this enormous subject of neurology and psychiatry are of greater help to the daily work but still giants of the present type offer much information to all of us

J C Melchior

G Joppich & H Wolf (eds) *Metabolism of the newborn child* Hippokrates Verlag Stuttgart 1969 448 pp illus DM 75—

Some European scientists convened for a symposium on the Metabolism of the Newborn Infant in Deidesheim Germany in October 1968. The present book is a wellwritten and most useful report from this meeting. The editors have had the initiative and courage to publish the different sections partly in German partly in English all depending upon the preference of the author

All the authors are established specialists and their combination of presenting new data and the main thoughts in the field at the same time is advantageously done. The chapters cover relationship between fetal and maternal metabolism, the metabolism of amino acids, proteins, fat and carbohydrates, the enzymatic and hormonal control of metabolism as well as a short section of inborn errors of disease

Gosta Roth

G Henneberg (ed) *Masernschutzimpfung* Abhandlung 11 Bundesgesundheitsamt, Heft 8 Springer Verlag Berlin Heidelberg and New York 1969 91 pp DM 22—

In USA vaccination with attenuated live measles virus vaccine has been practiced on a big scale for

many years. It has resulted in a marked reduction in morbidity and an apparently stable immunity. Side effects and severe complications have been rare. These American experience are incompletely reviewed in "Masernschutzimpfung". In this book mass prophylaxis against measles is not recommended. Inactivated measles vaccines which give less stable immunity and in some cases atypical severe reactions to live (wild or attenuated) virus is favored. In USA this type of vaccine is not recommended any more.

The current problem about the "slow virus infection" possibly resulting in progressive sclerosis, encephalitis is not touched upon. This book covers the subject incompletely which is especially noticeable for an official document.

Rutger Læeremans

P Krepler *Grundlagen und Fortschritte der Leukämiebehandlung beim Kinde* Beihefte zum Archiv für Kinderheilkunde No 62 Ferdinand Enke Verlag Stuttgart 1970 159 pp illus 18s DM 32.

During the last decade such a large number of investigations concerning childhood leukemia have been published that it is almost impossible for the ordinary pediatrician to keep up with the literature in this field. The book of Krepler gives a good review of the present situation of childhood leukemia.

Until 1963 the median life expectancy for patients with acute lymphatic leukemia was about 10 months. This was the result of prednisone and purine therapy and folic acid antagonists administered orally. Presently we possess about 10 drugs effective against acute lymphatic leukemia. There is however clinical experience only with a few combinations of these drugs. The best centers have now reached a median life expectancy exceeding 30 months.

A special chapter is dealing with immune therapy of leukemia. Although there are great practical problems in this field, definitely positive results have already been reached among others by Mithe and his associates in France. The immunotherapy seems to increase the number of cases with a definite cure of leukemia. There is still much research to be done and new therapeutic regimens are in progress. The review by Krepler which includes a large number of references possesses a great value for those who treat children with leukemia and for investigators in this field as well.

Eric Har Wilander

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ACKNOWLEDGEMENT

The Editorial Board of *Acta Paediatrica Scandinavica* wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers.

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ULTRASOUNDCARDIOGRAPHY IN INFANTS AND CHILDREN

NILS RUNE LUNDSTRÖM and INGE EDLER

*From the Departments of Paediatrics and Cardiology
University of Lund, Lund, Sweden*

Ultrasoundcardiography (UCG) was introduced by Edler & Hertz in 1954 (6). Since then the most important application of UCG has been the study of the movements of the anterior mitral leaflet for diagnosis and evaluation of the degree of mitral stenosis (5, 7, 12). The method has also been found useful to demonstrate the presence of pericardial effusion (2, 10, 16, 19), left atrial thrombosis (2) and tumours in the left atrium (8). The possibility of studying the movements of the anterior tricuspid valve in certain cases has also been reported (1, 3, 8, 15). Preliminary results of UCG in congenital heart disease with left to right shunting were presented by Jacoby *et al.* (14). Utan *et al.* (26) have more recently presented preliminary results of ultrasoundcardiography in congenital heart disease with particular reference to those with left to right shunting but also in cases with subaortic stenosis. Six children were included in this study but otherwise we have been unable to find publications on ultrasoundcardiography in infants and children. A systematic study of ultrasoundcardiography in infants and children, particularly in cases of congenital heart disease, has been commenced and we will present part of our results.

MATERIAL

The material consists of children without heart disease and children with various forms of heart disease. All age groups below 16 years of age (Table 1) are represented. The children without heart disease

were admitted to hospital for other causes than diseases affecting the heart or circulatory system. In these children the auscultatory findings of the heart and the electrocardiogram were normal. The children with heart disease were usually investigated with heart catheterization and angiocardiology during their stay in hospital. About one quarter of the material is comprised of infants below 1 year of age, the youngest being only a few hours old.

METHOD

Ultrasound examinations were carried out using an ultrasonoscope Smith Kline Eskoline 20. This instrument produces 1 000 pulses/sec and utilizes a 2.25 megacycle transducer of 0.75 inch diameter. The transducer acts as a sound transmitter for 1 μ sec and as a sound receiver for the remaining 999 μ sec until the subsequent pulse. The transducer is connected to a cathode ray tube. At each pulse the electron beam starts at the left side of the screen and moves to the right along the x-axis at a constant speed. Immediately after emission of each pulse the transducer becomes inactive and is mechanically excited by the returning echoes. These echoes are converted into an electrical signal which is then amplified and fed to the y-plates of the cathode ray tube. This results in a vertical deflection of the electron beam. The echo-signals are thus seen as vertical deflections on the oscilloscope screen (Fig. 1 A). The horizontal distance between the left border of the screen and the echo-signals is a measure of the time necessary for the pulse to travel from the transducer to a reflecting surface and back again. As the pulse rate from the transducer is 1 000 pulses/sec, the distance between the transducer and the reflecting surface is measured 1 000 times/sec.

As long as the velocity of the sound is the same in all the media it transverses, the horizontal distance between the vertical oscillations on the screen will be a measure of the distance between different reflecting surfaces. If the echo-giving structure moves towards the transducer, the echo-signal travels along the horizontal axis towards the left hand side of the

Table 1 Survey of material

Diagnosis	Age	No of patients
No heart disease	1 week-15 years	45
Congenital heart disease	1 day-16 years	224
Primary myocardial disease	2 weeks-15 years	12
Pleural effusion	5 months-15 years	4
Right atrial thrombosis	1 year	1
Total		286

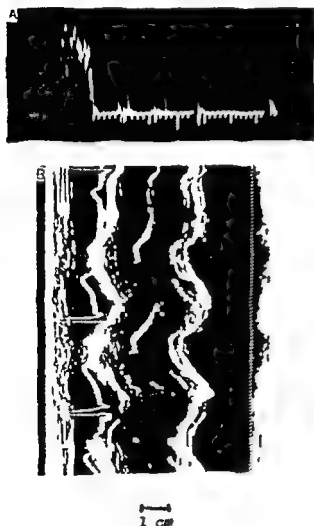


Fig 1 (A) Polaroid photographic picture of the oscilloscope screen as it is seen during scanning. The transducer was directed towards the aortic root (B) Photographic picture of one vertical sweep of the signals from Fig 1 A after the signals were converted into bright spots. An electrocardiogram was incorporated in the registration. If this picture is turned 90° clockwise the presentation is the same as in the following figures

screen. Alternatively if the echo-giving structure moves away from the transducer the echo-signal moves to the right hand side of the screen.

The movements of the signals cannot be directly recorded on film but are valuable for scanning the field and for choosing a desired echo. In order to record on a film the echo signals are transformed into bright spots along the invisible baseline. The strength of the echo is represented in this case by the brightness of the spot. The invisible baseline with the spots is swept upwards vertically on the screen. Using a Polaroid camera a photographic picture of the screen during one full sweep shows all horizontal movements in wave form (Fig 1B). A single lead electrocardiogram is incorporated in the recording. To facilitate measurements a time marker and calibration marks representing each centimeter are recorded.

Further by using a dual beam oscillograph with a four trace amplifier on one of the beams up to four different heart parameters such as electrocardiogram and phonocardiogram can be recorded simultaneously while the ultrasoundcardiogram is recorded by the other beam.

Another method used is the recording of the ultrasoundcardiogram via a direct writing electrocardiograph (9). The time used by the sound to travel to the reflecting structure and back (transit time) is converted to a voltage signal which can be recorded by the electrocardiograph. This conversion can be brought about by employing a condenser discharge for the measurements of the transit time. Since the ultrasonic impulses are being sent and received at the rate of 1000 per sec the resultant recording is essentially continuous. This technique permits recording of the movements of only a single echo but it is possible to correlate the ultrasoundcardiogram with simultaneously recorded electrocardiogram and phonocardiogram (Fig 6).

The patients were examined in a supine position during normal respiration. Most examinations were performed without premedication but a few anxious infants received a mild sedative. Severely ill infants were examined within an incubator.

RESULTS

Normal infants and children In all the cases examined we obtained echoes from the posterior wall of the heart and from the anterior mitral leaflet (Fig 2). The pattern of movement of the echo from the anterior mitral leaflet was similar to that observed in adults. These echoes were obtained with the ultrasonic beam in an *antero posterior* direction and with the transducer in the third or fourth left intercostal space and about half way between the left sternal border and the midclavicular line. During

the early part of diastole there is a rapid inflow of blood into the left ventricle. The echo from the anterior mitral leaflet makes a rapid posterior movement during this period. The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole was only measurable when the heart rate was slower than about 100 per min. At a higher heart rate the duration of the part of diastole before the atrial systole is too short to allow measurement of the speed of movement. The values for this speed of movement were between 90 and 140 mm/sec. Sometimes we observed an echo with a small amplitude of movement anterior to the echo from the anterior mitral leaflet. This echo probably arose from the interventricular septum.

Echoes from the anterior tricuspid leaflet were obtained more easily in infants and children than in adults without heart disease. These echoes could however only be obtained with the transducer directed in the medial cranial direction and placed in the fourth left intercostal space at the sternal border. The pattern of movement resembled that obtained from the anterior mitral leaflet but in most cases the echo was only fragmentary.

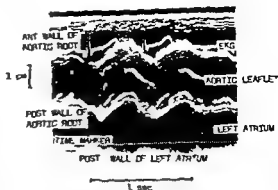


Fig 3 T 8 years of age. Normal aortic root (no heart disease). UCG of the root, the left atrium and the posterior wall of the left atrium. The recording is the same as Fig 1 B.

With the transducer in the second or third left intercostal space and directed medially or occasionally slightly cranially we observed two parallel echoes assumed to arise from the anterior and posterior border of the outflow tract of the left ventricle more caudally or the aortic root more cranially (Fig 3). Occasionally we observed a faster moving echo between the two parallel echoes in the more cranial direction. This echo moved towards one of the parallel echoes in the beginning of systole and away from it at the end of systole. It has earlier been demonstrated that this echo originates from an aortic leaflet (4). Posterior to the two parallel echoes we observed an echo-free space. The posterior border of that space consisted of an echo with a small amplitude of movement and with a movement in presystole identifying it as an echo from an atrial wall. This echo-free space has been identified as representing the left atrium (11, 13).

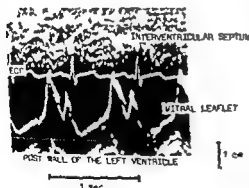


Fig 2 A B 10 years of age. Normal mitral valve (no heart disease). UCG of the anterior mitral leaflet and the posterior heart wall with simultaneously recorded electrocardiogram. As in all subsequent figures the anterior structures are in the upper part and the posterior structures in the lower part of the recording. The posterior movement of the echo from the anterior mitral leaflet during the early part of diastole is rapid (135 mm/sec).

Congenital heart disease with dilatation of the right ventricle. In children with various forms of congenital heart disease involving dilatation of the right ventricle we have always noted echoes from the anterior tricuspid leaflet and the interventricular septum. The echoes were obtained with the transducer in the fourth or fifth left intercostal space at the sternal border and the ultrasonic beam directed antero-posteriorly (Fig 4). The pattern of

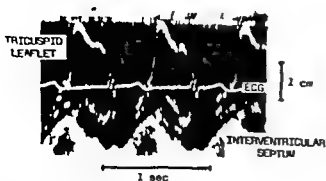


Fig 4 S W 13 years of age Atrial septal defect of secundum type UCG of the anterior tricuspid leaflet and the interventricular septum The recording was made with the transducer in the 4th left intercostal space at the sternal border and in antero posterior direction

movement of the echo from the anterior tricuspid leaflet corresponded to that obtained from the anterior tricuspid leaflet in adults This echo was in most cases observed continuously during both systole and diastole At a heart rate slower than 100 per min the speed of movement during the early part of diastole was measurable Excluding the cases of Ebsteins anomaly of the tricuspid valve the values for this speed of movement were between 55 and 160 mm/sec In some cases we observed echoes from two of the tricuspid leaflets The distance between the echo from the anterior chest wall and the echo from the interventricular septum was greater than normal for age in cases with dilatation of the right ventricle In the absence of dilatation of the right ventricle echoes from the anterior tricuspid leaflets were not obtained with antero posterior direction of the ultrasonic beam Echoes from the anterior tricuspid leaflet and the interventricular septum with an antero-posterior direction of the ultrasonic beam were noted in cases of atrial septal defects both of the ostium primum and secundum type the combination of atrial and ventricular septal defects very tight pulmonary stenosis Ebsteins anomaly of the tricuspid valve, primary pulmonary hypertension, some cases of ventricular septal defects combined with pulmonary stenosis a few cases of isolated ventricular septal defects and vari-

ous forms of cyanotic congenital heart malformations

Congenital heart disease with dilatation of the left atrium In cases with severe dilatation of the left atrium the distance between the echo from the anterior mitral leaflet and the echo from the posterior wall of the heart was greater than normal A greater distance than normal between the echo from the posterior wall of the aortic root and the echo from the posterior left atrial wall was also found in these cases

Congenital malformations of the tricuspid valve Tricuspid atresia was the only condition

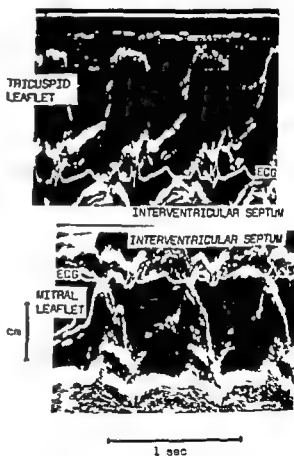


Fig 5 C S 6 years of age Ebsteins anomaly of the tricuspid valve UCG of anterior tricuspid leaflet and the interventricular septum (upper part) and the interventricular septum and the anterior mitral leaflet (lower part) Both recordings were obtained with the transducer in the 4th left intercostal space the lower part a little more to the left and both with the ultrasonic beam in antero posterior direction Note the abnormally anterior position of the echo from the anterior tricuspid leaflet during the entire diastole

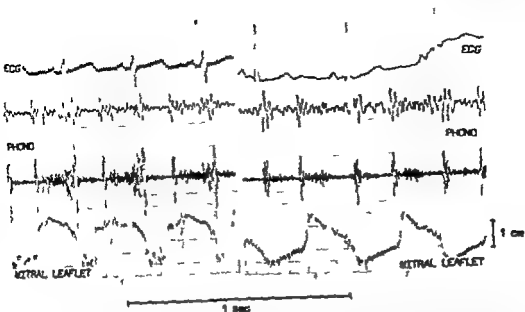


Fig 6 M P 3 years of age Congenital mitral stenosis and coarctation of the aorta UCG of the anterior mitral leaflet with simultaneously recorded electrocardiogram and phonocardiogram before and after valvotomy The recordings were made with a direct

writing electrocardiogram Note the abnormally slow speed of movement of the echo from the anterior mitral leaflet during the early part of diastole before valvotomy (25 mm/sec) and the increase in speed of movement after operation 40-45 mm/sec

in which we were unable to obtain any echo from the tricuspid leaflets

In 4 cases of Ebsteins anomaly of the tricuspid valve echoes from the anterior tricuspid leaflet and the interventricular septum were obtained with the ultrasonic beam in antero-posterior direction as in other forms of dilatation of the right ventricle The echo from the anterior tricuspid leaflet was however observed with the transducer placed more laterally on the precordium as far laterally as the left medioclavicular line The pattern of movement of the echo from the anterior tricuspid leaflet was during diastole characterized by a very slow posterior movement (< 25 mm/sec) This resulted in an abnormally anterior position of the echo from the anterior tricuspid leaflet during the entire diastole (Fig 5)

Congenital malformations of the mitral valve In 2 cases of congenital mitral stenosis both combined with coarctation of the aorta the pattern of movement of the echo from the anterior mitral leaflet had a distinctly abnormal

configuration (Fig 6) The total amplitude of movement of the echo was normal but the speed of movement in the early part of diastole was abnormally slow 20 and 25 mm/sec in the 2 cases After valvotomy the speed of movement in diastole increased to 35-45 mm/sec

In 2 cases of atresia of the mitral valve we observed echoes from the anterior tricuspid leaflet and the interventricular septum with the transducer in antero-posterior direction as in other cases with dilatation of the right ventricle Posterior to these echoes and 7-10 mm anterior of the echo from the posterior heart wall we observed an echo with a very small amplitude of movement (3-4 mm) its pattern of movement differed to that obtained from the mitral leaflet in all other cases (Fig 7) We have assumed that this echo represents the atretic mitral region In 4 cases with a hypoplastic left ventricle but with a mitral valve we also noticed echoes with a small amplitude of movement in front of the echo from the pos

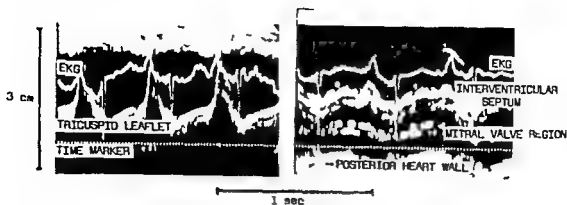


Fig 7 A L A 2 weeks old Congenital mitral regurgitation UCG of the anterior tricuspid leaflet (left part) and the interventricular septum the mitral valve region and the posterior heart wall (right part) Both recordings were made in the 4th left intercostal space

but left part closer to the left sternal border Note the abnormal echo obtained from the mitral valve region instead of the normal echo from the anterior mitral leaflet

terior wall of the heart In these cases, however the echoes from this region had a pattern of movement resembling that from a mitral leaflet In one of these cases we even obtained an echo from the posterior mitral leaflet there by confirming the existence of a mitral ostium

Four cases with atrial septal defect of the ostium primum type with cleft anterior mitral leaflet were examined In three of them we observed abnormal echoes from the mitral region Instead of the normal echo from the anterior mitral leaflet there were two parallel echoes both with a pattern of movement typical for the echo from the anterior mitral leaflet These echoes represented structures separated by a distance of 7 to 9 mm It was possible on angiocardiology in the lateral projection to demonstrate the two parts of the cleft anterior mitral leaflet The distance between the two parts measured on the angiocardiology was in close agreement with the distance between the two parallel echoes One patient has been operated upon and at inspection of the valve a cleft of the anterior mitral leaflet was found and sutured At postoperative investigations with UCG in this patient only a single echo from the mitral region was given

In 2 cases of severe congenital mitral regurgitation we found an abnormally large amplitude of movement of the echo from the anterior mitral leaflet compared with that in nor-

mal children of the same age The speed of movement during diastole was within the normal limits In two other cases with mitral regurgitation where the regurgitation was judged as slight we noticed normal echoes from the mitral region In another case of mitral regurgitation we found two parallel echoes from the mitral region in the same way as described in the 3 cases with a cleft anterior mitral leaflet and an atrial septal defect of ostium primum type The electrocardiogram of this patient was typical for a case of endocardial cushion defect but catheterization failed to prove any atrial septal defect A left ventricular angiogram demonstrated a deformed mitral valve and a mitral regurgitation but no cleft in the anterior mitral leaflet could be shown From our observations on UCG we suspect however that this patient has a cleft anterior mitral leaflet of the endocardial cushion defect type

We have investigated 3 cases of congenital corrected transposition of the great arteries with ventricular inversion In all three we observed two parallel echoes in the position normally held by the echo from the anterior mitral leaflet Both echoes had a pattern of movement typical for an echo from a leaflet of an atrio ventricular valve In this malformation there is a tricuspid valve between the left atrium and the arterial ventricle and a bicuspid valve between the right atrium and the venous

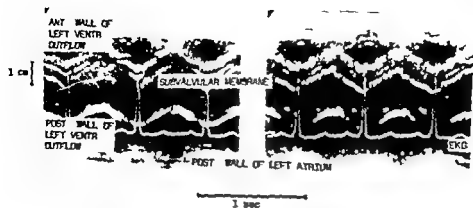


Fig 8 1 K 11 years of age Membranous subaortic stenosis UCG from the outflow tract of the left ventricle Note the abnormal echo from the subvalvular

lar membrane seen between the echoes from the anterior and posterior walls of the outflow tract of the left ventricle during both systole and diastole

ventricle We therefore assume that we registered echoes from two of the three leaflets of the tricuspid valve between the left atrium and the arterial ventricle

Congenital malformations with obstruction of left ventricular outflow Four cases of membranous subaortic stenosis have been studied In all of them we obtained an abnormal echo between the two parallel echoes from the outflow tract of the left ventricle (Fig 8) This echo was observed both during systole and diastole and was easily distinguished from the echo of an aortic leaflet With a slight angulation of the transducer we could obtain both the abnormal echo (more caudally) and an echo from an aortic leaflet (more cranially) In two of these cases the echo from the anterior mitral leaflet was normal but in the other 2 cases the speed of movement during the early part of diastole was slightly reduced (60-70 mm/sec) The pressure gradient between the left ventricle and the aorta was lowest in the cases with the normal echo from the anterior mitral leaflet

Several other cases with congenital obstruction to the outflow from the left ventricle (aortic stenosis isolated or combined with coarctation of the aorta) have been examined In 3 cases where the degree of obstruction was severe we noted an abnormal pattern of movement of the echo from the anterior mitral leaflet The speed of movement during the early

part of diastole was slower than normal (42-47 and 55 mm/sec) The echoes from the outflow tract of the left ventricle and the aortic root were normal

Primary myocardial disease Three patients with hypertrophic obstructive cardiomyopathy have been investigated They all showed an abnormal echo from the anterior mitral leaflet (Fig 9) The speed of movement during the early part of diastole was slower than normal

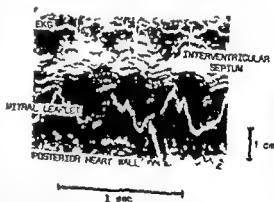


Fig 9 T M 2 years of age Hypertrophic obstructive cardiomyopathy UCG of the interventricular septum the anterior mitral leaflet and the posterior heart wall The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole is somewhat slow (75 mm/sec) Note the abnormal anterior movement of the echo from the anterior mitral leaflet (arrow) during ventricular systole and the broad echo from the interventricular septum

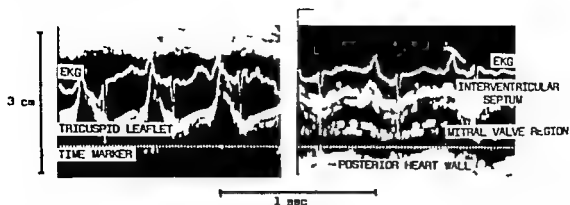


Fig 7 A L A 2 weeks old Congenital mitral stenosis. UCG of the anterior tricuspid leaflet (left part) and the interventricular septum the mitral valve region and the posterior heart wall (right part). Both recordings were made in the 4th left intercostal space

but left part closer to the left sternal border. Note the abnormal echo obtained from the mitral valve region instead of the normal echo from the anterior mitral leaflet.

terior wall of the heart. In these cases however the echoes from this region had a pattern of movement resembling that from a mitral leaflet. In one of these cases we even obtained an echo from the posterior mitral leaflet thereby confirming the existence of a mitral ostium.

Four cases with atrial septal defect of the ostium primum type with cleft anterior mitral leaflet were examined. In three of them we observed abnormal echoes from the mitral region. Instead of the normal echo from the anterior mitral leaflet there were two parallel echoes both with a pattern of movement typical for the echo from the anterior mitral leaflet. These echoes represented structures separated by a distance of 7 to 9 mm. It was possible on angiocardiology in the lateral projection to demonstrate the two parts of the cleft anterior mitral leaflet. The distance between the two parts measured on the angiocardiology was in close agreement with the distance between the two parallel echoes. One patient has been operated upon and at inspection of the valve a cleft of the anterior mitral leaflet was found and sutured. At postoperative investigations with UCG in this patient only a single echo from the mitral region was given.

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mal children of the same age. The speed of movement during diastole was within the normal limits. In two other cases with mitral regurgitation where the regurgitation was judged as slight we noticed normal echoes from the mitral region. In another case of mitral regurgitation we found two parallel echoes from the mitral region in the same way as described in the 3 cases with a cleft anterior mitral leaflet and an atrial septal defect of ostium primum type. The electrocardiogram of this patient was typical for a case of endocardial cushion defect but a catheterization failed to prove any atrial septal defect. A left ventricular angiogram demonstrated a deformed mitral valve and a mitral regurgitation but no cleft in the anterior mitral leaflet could be shown. From our observations on UCG we suspect however that this patient has a cleft anterior mitral leaflet of the endocardial cushion defect type.

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increased speed of movement of the echo from the tricuspid leaflet. This increased speed of movement was found to be reduced after operation. In our material the speed of movement of the echo from the anterior mitral leaflet in cases of ventricular septal defect and patent ductus arteriosus as well as that from the anterior tricuspid leaflet in cases of atrial septal defects was found to be within normal limits. Furthermore we have not been able to demonstrate a significant reduction after operation. Our material of these heart lesions studied by UCG is however rather small and larger material has to be investigated for an accurate evaluation of UCG in estimating the degree of left to right shunting.

Echoes from the tricuspid leaflet—with the transducer in antero-posterior direction—have only been found in cases with a dilatation of the right ventricle. It is not clear whether this is due to the dilatation per se or to a simultaneous rotation of the heart. For answering this a comparative study of UCG and right ventricular angiocardiology has to be performed. The UCG technique seems however promising as a screening test for detection of dilatation of the right ventricle.

In cases of Ebstein's anomaly of the tricuspid valve UCG has been found to be of particularly diagnostic value. The echo from the anterior tricuspid leaflet in this anomaly obtained far more laterally on the precordium than in other heart lesions with dilatation of the right ventricle and furthermore it has a different pattern of movement (17) resembling that described in cases of acquired tricuspid stenosis (1-15). There is however a difference in the pattern of movement of the echo from the anterior tricuspid leaflet in cases of tricuspid stenosis and in Ebstein's anomaly. In tricuspid stenosis the movement of the echo from the anterior tricuspid leaflet in diastole is influenced by atrial systole. In Ebstein's anomaly of the tricuspid valve the atrial systole causes no movement of the echo from the anterior tricuspid leaflet.

In all cases except tricuspid atresia at least

fragments of an echo could be obtained from the anterior tricuspid leaflet. In doubtful cases a typical echo from a tricuspid leaflet therefore excludes the diagnosis of tricuspid atresia.

Many cardiological centres have by now acquired considerable experience in the use of UCG for the diagnosis of mitral stenosis and evaluation of its severity. A fairly high correlation has thus been found between the degree of stenosis of the mitral valve and the speed of movement of the echo from the anterior mitral leaflet during the early part of diastole (5-7, 12). In 2 children with congenital mitral stenosis both combined with coarctation of the aorta the UCG findings are in agreement with those obtained in adults with acquired mitral stenosis. Moreover the increase in speed of movement of the echo from the anterior mitral leaflet after valvotomy corresponds to the findings in adults (4, 5, 12).

Mitral atresia is the only condition where we have not succeeded in obtaining an echo with the pattern of movement typical for that from the anterior mitral leaflet. Instead an abnormal echo was obtained with a pattern of movement similar to that described in normal subjects and patients with acquired mitral disease. By Zaky et al. (27) they assumed that this echo originates from the mitral ring region. This observation together with the UCG findings in other cases of the so-called hypoplastic left heart syndrome indicate a new adjunct in the methods of diagnosing this group of congenital heart disease and particularly mitral atresia.

Sommerville (24) has pointed out that mitral regurgitation is one of the important factors determining the natural history of endocardial cushion defects. Demonstration of an abnormal mobility of the mitral leaflets and of a cleft anterior mitral leaflet are important for the diagnostic evaluation of cases with atrial septal defect of the ostium primum type. UCG can therefore contribute to a better understanding of the function of the mitral valve in these cases. The prerequisite for obtaining double echoes from a cleft mitral leaflet is of course that the two parts of the leaflet are

(40, 70 and 75 mm/sec) In one of these patients showing a speed of movement of 70 mm/sec a septomyotomy was performed and after the operation the speed of movement increased to 85 mm/sec The most important abnormal finding in these patients was however, an anterior movement of the echo from the anterior mitral leaflet during ventricular systole, thus narrowing the outflow tract of the left ventricle This anterior movement was most pronounced in the case with the greatest pressure gradient between the left ventricle and the aorta This abnormal movement could best be seen by registration of the echo from the anterior mitral leaflet in the usual way It could however also be seen at least in the most severe cases when the transducer was directed towards the lower part of the outflow tract of the left ventricle The echo from the interventricular septum was unusually broad in these cases with hypertrophic obstructive cardiomyopathy

In 3 cases with severe acute myocarditis normal echoes from the anterior mitral leaflets were registered except in one of the patients In the initial stage with severe congestive heart failure this patient showed a slightly reduced speed of movement of the echo from the anterior mitral leaflet during the early part of diastole (75 mm/sec) but this later became normal

Pericardial effusion In 4 cases with pericardial effusions an echofree zone between the echo from the anterior structures presumably also including the parietal pericardium, and that from the anterior heart wall was found In two of them an echofree zone posterior to the echo from the posterior heart wall was also found After pericardiocentesis the echofree zones disappeared

Atrial thrombosis In 1 patient abnormal echoes were found posterior to the echo from the anterior tricuspid leaflet and anterior to the echo from the posterior heart wall These registrations were made with the transducer in the fourth left intercostal space parasternally and placed in medio cranial direction This

space posterior to the echo from the anterior tricuspid leaflet was later confirmed to be the right atrium by obtaining dense echoes in this area at injection of indocyanine green using the method described by Gramiak et al (11) The abnormal echoes obtained without contrast injection were stratified and moved in and out of the ultrasonic beam with each heart beat Angiocardiography confirmed the presence of a mass presumably a thrombosis, in the posterior part of the right atrium This patient had earlier been operated upon twice with insertion of Spitz Holter catheters for hydrocephalus These catheters had been removed because of malfunction

DISCUSSION

Principally the same structures as in healthy adults can by UCG be studied in infants and children without heart disease Echoes from the anterior tricuspid leaflet are however more easily obtained in younger individuals than in adults As in adults a complete UCG study of the mitral valve requires a sufficiently long interval between the end of ventricular systole and the beginning of atrial systole The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole was measured to 90–140 mm/sec i.e. the same values as in adults (3)

In cases of atrial septal defect ventricular septal defect and patent ductus arteriosus Jacob et al (14) found for each defect a typical pattern of movement of the echo from the anterior mitral leaflet Like Schmitt et al (20) we have been unable to confirm this observation

In cases of ventricular septal defect and patent ductus arteriosus with left to right shunting Ultan et al (26) found during the early part of diastole a more rapid movement of the echo from the anterior mitral leaflet than in healthy subjects This has been attributed to an increased flow through the mitral ostium In cases of atrial septal defects with left to right shunting the same authors found a similarly

movement of the echo from the anterior mitral leaflet during ventricular systole and on the other the systolic murmur and the configuration of the arterial pressure curve in these cases as well as the effect of pharmacological tests on all these parameters UCG therefore seems well established as a diagnostic tool in cases of hypertrophic obstructive cardiomyopathy and in addition it provides valuable information on the variability of the outflow obstruction in this disorder

The usefulness of UCG for demonstration of pericardial effusion has previously been reported by many authors (2 9 16 19) Our observations confirm this and thus show that the UCG technique is for this purpose also applicable on infants and children

Several authors have reported that tumour or thrombosis can give abnormal echoes (2 8) most of these have been demonstrated from the left atrium Our UCG studies in a patient with thrombosis in the right atrium show that information may also be obtained from the right atrium This examination is especially valuable in patients operated for hydrocephalus with the Spitz Holter method

Pronounced deformity of the thoracic wall makes the use of UCG difficult due to the need for air free contact between the transducer and the chest wall The use of a smaller transducer can solve some of these problems Anomalies of the position of the heart makes the interpretation of the UCG difficult the use of the contrast method as described by Gramiak et al (11) facilitates however the identification of echoes and echofree spaces within the heart

The danger of tissue damage from the use of ultrasound for diagnostic purpose has been discussed by several authors (3 23) No tissue damage has however so far been discovered following investigations with reflected ultrasound in the form used here This is also unlikely to happen due to the small amount of energy produced by this technique with its very short pulses of ultrasound

One of the advantages of the UCG tech-

nique is that it offers a simple and fairly rapid examination which can easily be repeated It can even be used in small seriously ill infants also these lying in an incubator The method is of great value as a complement to the ordinary methods for investigation of infants and children with heart disease

SUMMARY

Ultrasoundcardiography (UCG) studies have been performed in about three hundred children The age of the children varied between 1 day and 16 years Most of the studies have been performed without premedication and all without discomfort

The UCG technique has been found valuable for demonstration of dilatation of the right ventricle both in cyanotic and acyanotic congenital heart disease In studies of lesion affecting the tricuspid valve pathological UCG findings have been obtained in patients with tricuspid atresia and Ebsteins anomaly

The method has also been found useful in evaluation of the state of the mitral valve Pathological findings have been obtained in congenital mitral stenosis mitral atresia and other forms of hypoplastic left heart syndrome in endocardial cushion defects and in mitral regurgitation

The UCG technique also provides information about the outflow tract of the left ventricle Pathological findings have been obtained in cases with membranous subvalvular aortic stenosis and hypertrophic obstructive cardiomyopathy

Pericardial effusion and thrombosis in the right atrium may be demonstrated by this technique No complications have been observed

ACKNOWLEDGEMENT

This work was supported by a grant (to N R L.) from the Swedish National Association against Heart and Chest Diseases

located at different distances from the anterior chest wall. On the other hand it must be stressed that the finding of double echoes from the mitral region is not synonymous with a cleft mitral leaflet, this has clearly been demonstrated in our studies of patients with congenital corrected transposition of the great arteries with ventricular inversion. In cases with abnormally thick mitral leaflets a double echo from the anterior mitral leaflet can sometimes be found. In these cases is, however, the distance between the two parts less than that obtained in cases with cleft anterior mitral leaflet.

Our observations in patients with severe mitral regurgitation of a large amplitude of movement of the echo from the anterior mitral leaflets are in agreement with the UCG findings reported in cases of acquired mitral regurgitation (5, 21).

The echofree zone between the echo from the anterior mitral leaflet and that from the posterior heart wall has been assumed to represent the left atrium in most cases (4). This has by means of contrast studies now been confirmed by Gramiak et al (11). In cases of severe dilatation of the left ventricle and with a more caudal direction of the ultrasonic beam this zone will instead represent the left ventricle. These two possibilities can easily be differentiated from each other by noting the different pattern of movement of the echo from the left atrial wall as compared with that from the left ventricular wall: the echo from the posterior left atrial wall moves in an anterior direction during presystole while the echo from the posterior left ventricular wall moves in an anterior direction during systole. The echofree zone posterior to the posterior wall of the left ventricular outflow tract or the aortic root gives, however, a more reliable presentation of the left atrium and permits also a rough estimate of the size of the left atrium. A more accurate measurement may however be made by the technique suggested by Hirata et al (13).

Ullman et al (26) have described an echo presumably representing a membranous sub

aortic stenosis and they have also presented certain criteria for the identification of this echo. In such cases we have observed a similar echo within the outflow tract of the left ventricle. This echo does not resemble that from an aortic leaflet, the latter is most often seen between the two parallel echoes from the aortic root. The UCG findings in patients with membranous subaortic stenosis are also different from those obtained in cases of hypertrophic obstructive cardiomyopathy. We would however, like to add one more criterion to those given by Ullman et al (26), the echo from a membranous subaortic stenosis must be identified between the two parallel echoes from the outflow tract of the left ventricle or be anterior to the echo from the anterior mitral leaflet, and, furthermore, clearly separated from the echo from the interventricular septum. If these criteria are not fulfilled the echoes from the mitral ring region or the interventricular septum may be misinterpreted as coming from a subvalvular membrane.

A reduced speed of movement of the echo from the anterior mitral leaflet during the early part of diastole has been demonstrated in cases of severe valvular aortic stenosis (5). This is presumably due to an impaired inflow to the left ventricle secondary to a reduced compliance. The same mechanism is presumably also responsible for similar findings obtained in patients with hypertrophic obstructive cardiomyopathy. Stewart et al (25) have demonstrated in patients with hypertrophic obstructive cardiomyopathy and valvular aortic stenosis a reduced compliance of the left ventricle.

The anterior movement of the echo from the anterior mitral leaflet observed during ventricular systole in cases of hypertrophic obstructive cardiomyopathy seems to be typical for this disease. This abnormal movement of the anterior mitral leaflet explains probably the mitral regurgitation often seen in these cases. Similar observations have recently been published by Shah et al (22) and Pridie et al (18). Shah et al (22) have clearly demonstrated the relation in time between on one side the an

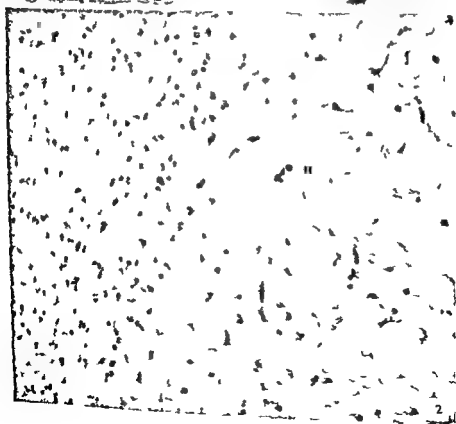


Table 1 Laboratory data of case II

	Age (hours)									
	72	90	98	118	138	144	146	162	174	
Serum K (mEq/l)	8.2	9.1	6.9	7.4	4.8	5.0		6.2		
Serum Na (mEq/l)	131	139	139	134	130	132		142		
Serum Cl (mEq/l)	97	98	93	92	85	77		102		
pH	7.00	7.00	7.09	7.04	7.04	7.03	7.12	7.38	7.19	
St. bicarbonate (mEq/l)	8	8	10	12	14	14	16	17	14	
Pco (mmHg)	32	30	30	59	85	70	58	22	36	
O ₂ saturation ()			96	98				100		
Urea (mg/100 ml)		99		165	199			147		
Creatinine (mg/100 ml)					4.4					
Blood glucose (mg/100 ml)	180									
Serum C ₁ (mEq/l)		4.2			4.3				2.6	
Serum Mg (mEq/l)					1.8				1.5	
Prothrombin test ()				15						
Albumin (g/100 ml)					4.0					
Bilirubin (mg/100 ml)					4.4					
SGOT (U/l) (normal 4-20)		71								
SGPT (U/l) (normal 2-17)										
LDH (U/l) (normal < 200)		1 800			2 300					
HBDH (U/l) (normal < 125)		1 380			1 615					
CPK (U/l) (normal < 35)		660			296					
Hgb (g/100 ml)	15.5									
WBC (No per μ l)	11 400									
Lactic acid (mg/100 ml blood)			97	88			97		109	
Pyruvic acid (mg/100 ml blood)				3.3						

Laboratory investigations (68 hours of age) Hgb 18.8 g per 100 ml RBC 5.26 millions per μ l hemato crit 60 WBC 30 200 per μ l nucleated red cells 3 per 100 WBC thrombocytes 169 000 per μ l prothrombin test 10 blood sugar 30 mg per 100 ml Serum electrolytes (mEq/l) potassium 8.2 sodium 134 chlorides 113 calcium 4.4 Acid base values pH 7.04 standard bicarbonate 9 mEq/l PCO₂ 22 mmHg Spinal puncture yielded a yellow cloudy fluid with a cell count of 1 per μ l spinal fluid glucose 13 mg per 100 ml spinal fluid protein 288 mg per 100 ml

The clinical picture was consequently that of a severe metabolic acidosis with elevated serum potassium levels. A septic etiology could not be excluded and the child was given penicillin sulphonamides and chloramphenicol. There was no response to the treatment. The acidosis was persistently severe and the serum potassium level remained high. Increasing spasticity and signs of cerebral degeneration characterized the development. Determinations at 94 hours of age showed serum potassium at 8.1 mEq/l sodium 138 mEq/l chloride 92 mEq/l pH 7.01 standard bicarbonate 11 mEq/l and PCO₂ 35 mmHg. A severe secedema developed and the child expired at the age of 100 hours. No examinations were made in order to find organic acids in the serum which could explain the acidosis.

Case II I L S (born April 2 1968)

The pregnancy was uneventful with delivery 20 days after term of a girl with birth weight 3 480 g length

55 cm. The child cried immediately and appeared healthy. Placenta was normal. During the second day of life the child had some vomiting and appeared slightly dehydrated. On April 5th the child was admitted to our department.

On admission the child was nearly 72 hours of age. She had a dysmatur appearance with loose wrinkled skin. Truncus was long and thin. There were definite signs of cerebral damage including hypertonicity and high pitched cry. The respiration rate was somewhat increased (48/min) and on auscultation some coarse rales could be heard over both lungs.

The results of laboratory investigations are presented in Table 1. Spinal puncture revealed a yellow fluid with a cell count of 3 per μ l spinal fluid glucose 40 mg and spinal fluid protein 218 mg per 100 ml. Bacteriological examination of the spinal fluid was negative. Amino acid chromatography of urine and serum did not reveal any significant deviations from the normal picture. The urine was acid and contained protein as well as ketone bodies.

According to Table 1 the child suffered from a severe metabolic acidosis with an elevated serum po-

Fig. 1 Case I O B S Section through cerebellum showing bilateral cystic spaces protruding into the hilum of the dentate nuclei $\times 16$ Weil staining.

Fig. 2 Case I O B S Cerebral white matter transition to gelatinous encystic area. Loose spongy structure with few cells $\times 160$ Luxol fast blue.

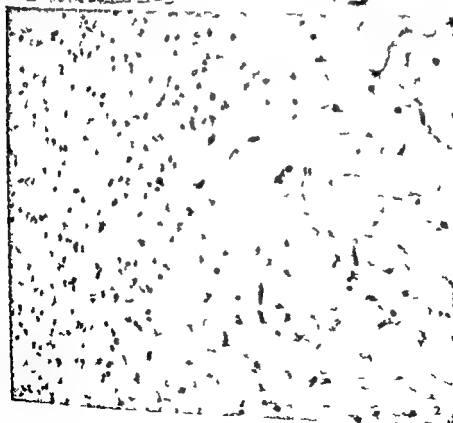


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Fig. 1 Case I O B S Section through cerebellum showing bilateral cystic spaces protruding into the hilum of the dentate nuclei. $\times 16$ Weil staining.

Fig. 2 Case I O B S Cerebral white matter transition to gelatinous encystic area. Loose spongy structure with few cells. $\times 160$ Luvol fast blue.

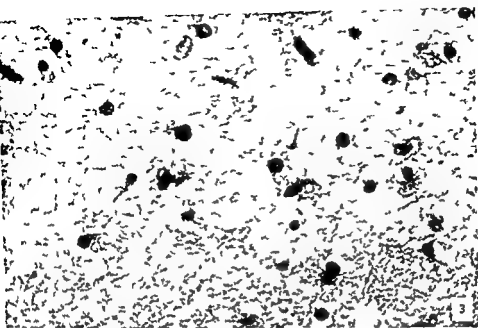


Fig 3 Case I O B S Swollen cytoplasm in glial cells rounded or stellate $\times 640$ Luxol fast blue

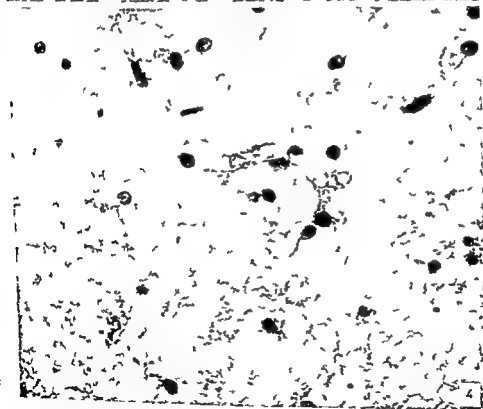


Fig 4 Case I O B S Pale staining stellate glial cells in spongy semicystic area $\times 640$ Luxol fast blue

tassium. Calculation of the available electrolyte values suggested an accumulation of some organic acid in the blood. Lactic acid and pyruvic acid showed 88–108 mg lactate per 100 ml of blood (9.8–12 mMol per l) and 3.3 mg pyruvate per 100 ml (0.4 mMol per l). A ratio of lactic acid to pyruvic acid of 24 to 1 was found. Gas chromatography of urine showed lactate values from 68 to 434 mg per 100 ml. The diagnosis of a severe lactic acidosis was thus confirmed.

Treatment was started with antibiotics (penicillin, streptomycin and colimycin) and intravenous fluid including isotonic bicarbonate. The acidosis persisted and even rapid injections of hypertonic bicarbonate did not have any significant effect on blood pH.

The elevated level of serum potassium was confirmed by repeated determinations (Table 1). Reduction of the high levels was achieved by the combined use of resonium by mouth and by adding glucose with insulin to the intravenous program. Glucose was the only source of calories given to the patient the first days after admission. However, as the need for parenteral nutrition persisted, amino acids were given together with lipids (Intralipid®). Neither of these regimen seemed to influence the production of organic acids in the body.

The general condition of the infant deteriorated rapidly and sclerodema became obvious. Cerebral damage became more pronounced and from the fourth day of life the child presented with no motor activity except for respiration. An exchange transfusion was performed on April 8th as a last attempt to alleviate the acidosis and to clear the body of the accumulating metabolites. During this procedure however the child got apnoea and had to be put on a respirator (age 144 hours). The acid base values showed an improvement after this, however with a pH the next day of 7.38, standard bicarbonate 17 mEq/l and PCO_2 22 mmHg. However the clinical condition remained unchanged. At the age of 177 hours the child died from acute occlusion of the airways.

Autopsies

The pathological findings in the two siblings were nearly identical and restricted to the lungs, the liver, the thymus and the brain. They are here described together.

The lungs were atelectatic with small hemorrhages.

The liver had a yellowish brown colour, size and consistency were not remarkable. Microscopically a few hematopoietic foci occurred. The liver cells were vacuolated and frozen sections from the second case showed them filled with Sudanophilic material (tissue for frozen sections from visceral organs of case I was not

preserved). Paraffin embedded sections showed PAS positive loading in the liver cells, mainly around the central veins.

The kidneys were normal, but in frozen sections from case II Sudanophilic material was also found in the epithelial cells of convoluted tubuli.

The thymus was in both cases small (weight 5 g and 2.5 g). Cortex was nearly depleted of lymphocytes, and contained large cells filled with Sudanophilic droplets and also PAS positive granules. These changes were mostly marked in the second case.

The brain (weight 550 and 530 g). One hemisphere from case II was immediately after removal preserved deep frozen, otherwise the brains were fixed in 10% neutral formalin for about 14 days before cutting. The hemispheres were symmetrical and the gyri well developed. The consistency was soft. Coronal sections showed widening of the lateral ventricles. Subependymal cystic spaces were seen lateral to the side ventricles and separated from them only by a thin membrane. Similar cystic spaces were seen also in the cerebellum localized to the hilum of the dentate nuclei (Fig. 1). The cut surfaces showed white to greyish gelatinous sunken areas mainly localized to the central parts of the white matter, mostly pronounced in frontal and temporal regions. Microscopically the subependymal cysts were lined by a few layers of organized glial cells. The ependymal lining of the ventricles was well preserved. The cortex and central gray substance showed a development as expected in newborn infants. The white substance and especially the central parts had a spongy structure (Fig. 2). It was poor in cells and those present were stellate astroglial cells with varying amounts of visible cytoplasm which in frozen sections contained small Sudanophilic granules (Figs. 3 and 4). These were more numerous in the better preserved periventricular areas and absent in the looser gelatinous parts. Rounded eosinophilic cells were also conspicuous. PAS staining of the brain was negative.

Inflammatory cells were not present in any

Clinical course

Died 3½ years of age

Improved

Died 2 years of age

Improved
Improved

Died 1½ years of age

Died 2 years of age

Died 16 months of age

Observed till 7 months

Died 7 months of age

Observed till 4 years of age

Died 30 months of age

biochemical defects. No enzymatic deficit has so far been demonstrated in any patient.

We feel that the 2 patients described in the present report constitute a further subgroup of chronic lactic acidosis as they differ in several respects from those previously reported.

Clinical course The disease manifested itself during the second and third day of life. The early signs were slight dehydration, irritability and signs of cerebral damage. Convulsions were never observed. The cerebral damage was reflected through an increasing hypertonicity and elimination of spontaneous movements of the extremities. Death occurred at the age of 100 and 174 hours.

One of the common signs among the children with lactic acidosis has been episodes of hyperpnoea associated with rise in blood lactate. Our patients might have had a slight hyperventilation early in life indicated by the low PCO₂ detected in the first patient on admission. However, no real respiratory effort to get rid of the acid load could be detected clinically and the PCO₂ values increased with

time. One explanation of this could be a damage to the respiratory center along with the general cerebral damage that obviously progressed as the metabolic acidosis persisted. None of the previously reported cases of hyperlactatemia of infancy has showed such a malignant course.

Biochemical findings Serum potassium levels were elevated in both patients. In the children previously described where this has been mentioned, normal values have been found. A possible explanation of this elevation could be renal failure, although renal function was not markedly reduced early in the disease. The acidosis could also explain some of the elevation as an accumulation of H⁺ ions can cause a shift in potassium from the intracellular to the extracellular phase.

Aminoaciduria could not be detected in our patients but has been described by others (6, 9, 20). Those who have examined the spinal fluid have found normal liquor. Both our patients had a marked increase in spinal protein. Even though the protein content of the spinal fluid normally is somewhat elevated in the neonatal period, the elevation demonstrated was clearly pathological. The increase is compatible with a process of severe damage to nervous tissue.

The level of lactic dehydrogenase (LDH) was greatly elevated in the serum of the second sibling. This increase has been found in children with congenital lactic acidosis (9) as well as in the idiopathic acidosis of adults (17). The isoenzyme pattern suggests that the excess LDH is of hepatic origin (16). The levels of creatine phosphokinase were elevated as well in the second sibling. This could point to muscle damage but can also be seen where there is destruction of nerve cells. Details of biochemical investigations performed on the second patient can be found in the accompanying paper (16).

Autopsy findings The changes were close to identical in the two siblings. Both livers were heavily stored by lipid and some lipid was also disclosed in the tubular epithelium in the kid-

Table 2 Chronic lactic acidosis in children Data on previously reported cases

Authors	Sex	Birth weight	Age at start of hyperpnoea	Age at diagnosis	Hypotonicity	Convulsions	Mental retardation	Serum potassium	CSF ^a
Hartmann et al (1962)	♀	2 600	Early infancy	4 months	+	+	+	N ^b	?
Nordio et al (1963)	♂	3 000	10 days	16 days	?	+	—	?	?
Israels et al (1964)	♀	?	8 months	11 months	+	+	+	?	?
	♂	2 100	4 months	9½ months	+	+	+	N	N
Erickson (1965)	♂	3 530	1 day	3 weeks	+	+	+	N	?
	♀	?	1 day	17 months	+	+	+	N	?
Worsley et al (1965)	♂	3 175	18 months	17 months	+	+	+	N	N
	♂	3 855	4 months	19 months	+	+	+	N	N
Haworth et al (1967)	♂	?	3 months	3 months	+	—	+	N	N
	♂	3 840	1 day	3 days	+	+	+	N	?
Schärer et al (1968)	♂	2 850	8 days	5 months	+	+	+	N	N
Bejar et al (1968)	♂	?	Never had	4 years	—	—	—	?	?
Peytel et al (1969)	♂	2 900	First months of life	8 months	+	+	+	N	N

^a Cerebrospinal fluid^b Normal values

part examined. Myelin stained sections showed no myelin in the hemispheres, the internal capsules or in the cerebellum. Around the basal ganglia and brain stem a few myelinated fibers were present. The spinal cords revealed faintly staining myelin in the posterior columns and ascending sensory tracts. The root fibers all stained well and could be followed to their nuclei in the cord and brain stem. The axillary cylinders were scarce and appeared fragmented in the unmyelinated hemispheres.

DISCUSSION

Two siblings have been described who developed a severe metabolic acidosis during the first days of life. In the second sibling the acidosis was demonstrated to be caused by an accumulation of lactic acid in the serum. In the first sibling no lactic acid determinations were made. However, the similarities in clinical picture, the almost identical biochemical disturbances and autopsy findings combine very

strongly to suggest an identical condition. The fact that they were siblings suggest that genetic factors are of importance. As to the etiology of the hyperlactatemia we were unable to disclose any of the known conditions which may give lactic acidosis (see p 124). We therefore consider our two patients to suffer from a primary type of lactic acidosis in which biochemical lesion most probably is present.

Thirteen patients have so far been described in the literature with chronic hyperlactatemia in early infancy and childhood. Some of the clinical and biochemical features of these patients have been summarized in Table 2. The common denominator in all has been the demonstration of a chronic metabolic acidosis caused by an accumulation of lactic acid in the serum. In three of the reports (2, 6, 20) the patients described have been siblings, suggesting a genetic basis for the disease. The clinical course in the individual patients, however, has varied considerably, suggesting that the hyperlactatemia can be caused by different

mination of lactic acid was performed but the similarities in clinical biochemical and pathological findings combine very strongly to suggest an identical condition

The children died at the age of 100 and 177 hours. The main pathological findings were confined to the brain: the thymus and the liver. Softening of the white matter was pronounced with delayed myelination. Organized bilateral paraventricular cystic spaces were found. Fatty changes were pronounced in the liver.

In some respects these children differ from those previously reported with congenital lactic acidosis.

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neys where frozen sections were examined. Whether this storage can be connected with the metabolic disturbance possibly responsible for the cerebral damage cannot be decided.

Both patients had small thymus glands with few lymphocytes and many macrophages containing lipid and mucoprotein material. As no immunological study was possible the question of the role of the thymus gland in this context must also be left open.

The immediate impression gained on gross examination of the brain was the similarity to the picture described as sway back in lambs. Winkelman & Moore (19) found the same likeness in the brain from a 12 week old child in whom a metabolic disturbance had not been considered. Their diagnosis was a degenerative encephalopathy.

Our 2 cases had only lived for a few days, and myelin formation is at that time only starting in the hemispheres. Compared to the normal pattern, however, our cases were definitely behind in development. In both cases the subependymal cysts showed an organization in their walls that pointed to a process started during the intrauterine period. No signs of hemorrhage could be found. There were no reasons for assuming anoxia to have caused the lesions since both pregnancy and delivery were uncomplicated. The cortex was well organized, which gives us reason to presume that whatever had interfered had acted during the last 2 months. Considering the importance of oligodendroglia in the formation of myelin it seems justified to locate the deficient process to these cells.

Worsley et al (20) described two siblings with lactic acidosis, one of whom died at the age of 24 months. They found changes in both the grey and white matter, the first mainly located to diencephalon, reticulate substance and the brain nerve nuclei. They labelled their cases as necrotising encephalopathy. Even considering the different development in their case and ours, it is difficult to imagine a similar distribution of lesions if our patients should have survived.

Scharer et al (15) described 1 case of lactic acidosis living for 7 months. Their findings are more compatible with the present cases with lack of myelin in frontal parts of the brain, the internal capsules and corpus callosum as well as a marked subependymal gliosis in pons, cerebellum and around the 3rd ventricle. They also found changes in cerebellum although myelin was present. Peytel et al (13) reported 1 case of lactic acidosis living for a few months. They found bilateral ventricular dilatation and frontal atrophy. A pallidal necrosis was ascribed to intoxication by CO occurring as a consequence of serious acidosis. Their findings may be in conformity with ours as the process not necessarily must work with the same speed in all cases.

Poser (14) discussed two types of dysmyelinating disease: the myelinoclastic and the dysmyelinogenic. In the first, myelin is destroyed by exogenous or endogenous factors; in the second, group myelin is not produced due to various enzymatic deficiencies. He also pointed to the differences in myelin of the peripheral and central nervous system and the possibility of different types of myelin even within the brain that might be responsible for the variable location of lesions in different reported cases. We do not think it is possible to determine whether enzymatic defects or toxic substances are the cause of the deficiency in myelin observed in our patients. However, attention should be drawn to 1 case of fructosemia (10) where the pathological findings were quite similar to those presented in our 2 patients. Although the enzymatic defects are not identical, an interference with glial metabolism may have resulted in similar morphological picture.

SUMMARY

Two siblings have been described who developed a fatal metabolic acidosis during the first days of life. The acidosis was found to be caused by an accumulation of lactic acid in the second sibling. In the first sibling, no deter-

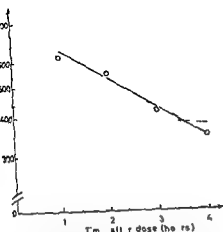


Fig. 1 Serum radioactivity after the injection of lactate ^{14}C . The stippled curve represents the calculated decay curve caused by metabolism (See the text for further details)

Estimation of glycogen The glycogen content was determined essentially by the method of Hultman (16). Glycogen standards were carried through the whole procedure in each separate analysis.

The radioactivity incorporated into glycogen during the *in vivo* as well as in the *in vitro* studies was determined upon its isolation (16).

Estimation of LDH isoenzymes Electrophoresis was performed in agarose gel (1%) in barbital buffer pH 8.6 and the fractions were visualized according to Barnett (3). The relative distribution of the isoenzymes was calculated from values obtained by scanning.

Incubation experiments Liver and muscle (diaphragm) slices of 0.5 mm thickness were made with a Stadie Riggs microtome. As incubation medium was used a Krebs-Ringer phosphate buffer pH 7.4 containing in mM: NaCl 127, KCl 5.10, CaCl₂ 2.73, MgSO₄ 1.77, KH₂PO₄ 11.2. To 3 ml of the buffer in a 50 ml Erlenmeyer flask were added 0.3 to 0.5 g of the tissue slices and 10 μmoles of 1- ^{14}C lactate or 1- ^{14}C pyruvate. The flask was stoppered and incubated in a shaking water bath at 37°C for 1 hour. After incubation 0.5 ml of 1N HCl was added and the ^{14}C was trapped in 1 ml of Hyamine hydroxide in a liquid scintillation counting vial.

Liver mitochondria were prepared by homogenization in 0.25 M sucrose with 0.5 mM EDTA and centrifugations according to Myers & Slater (13). The oxygen uptake was measured with conventional Warburg techniques.

RESULTS

In vivo studies

After the *in vivo* injection of lactate 2- ^{14}C (about 5 μCi) a nearly exponential disappearance of

the serum radioactivity was found the rate constant of which was about 0.23/hour ($T/2 = 3$ hours) (Fig. 1).

Only insignificant radioactivity was recovered in the urine in 4 $\frac{1}{2}$ hours (Table 1) amounting to a total of about 0.45% of the injected dose. The excretion was considerably lower during the first hour after the injection than in the following 3 hours despite the fact that the serum radioactivity decreased exponentially during the study (Fig. 1). This suggests that urinary lactate derived from tubular excretion rather than glomerular filtration.

Table 2 shows the amounts of radioactive ^{14}C expired in periods of 5 min at 1, 2, 3 and 4 hours after the injection of lactate 2- ^{14}C . From these values it can be estimated that

Table 1 Urinary excretion of lactate and ^{14}C lactate

Sample period (hours after dose)	Lactate excretion (mg/hour)	Radioactivity	
		(cpm/hours)	(cpm/mg lactate)
0-1	1.69	370	220
1-3	3.40	13 200	3 880
3-4 $\frac{1}{2}$	3.56	9 660	2 720

Table 2 Expiratory $^{14}\text{CO}_2$ and CO_2

Time of sampling (hours after dose)	$^{14}\text{CO}_2$ expired (counts/5 min)	CO_2 expired (mmoles/5 min)
0	0	4.1
1	12 570	3.6
2	19 300	4.0
3	19 850	4.3
4	15 350	3.6

Table 3 Radioactivity in various tissues post mortem

Tissue	Radioactivity (cpm/g tissue w/w)
Liver	2 780
Muscle	2 520
Heart	2 780
Brain	2 160
Kidney	3 000
Serum	2 200

FATAL CONGENITAL LACTIC ACIDOSIS IN TWO SIBLINGS

II Biochemical Studies *in vivo* and *in vitro*

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A preceding paper (19) dealt with the results of clinical investigation and autopsy findings in two newborn siblings with congenital lactic acidosis. Thirteen similar cases have been described till now (4, 8, 10, 12, 17, 24, 25, 29, 33), but a common cause of the accumulation of lactic acid has not been revealed. The present report deals with *in vivo* as well as *in vitro* studies carried out in an attempt to localize the metabolic defect in one of the children.

METHODS

Chemicals: Lactate 1^1C , pyruvate 1^1C and lactate 2^1C were obtained from the Radiochemical Center, Amersham, England. Caprinylcarnitine was a gift from Dr Jon Bremer. All other reagents were commercial products of high purity.

Radioactivity assays: The radioactivity was measured in a Tricarb liquid scintillation counter. The counting efficiency for ^1C varied from 40 to 55%, but all the values presented are deduced to the same counting efficiency.

In vivo studies

At the age of 7 days, a single tracer dose of $10 \mu\text{Ci}$ L-lactate 2^1C was given intravenously. Blood samples were taken at intervals. Urine was quantitatively collected and expiratory CO_2 was sampled as described below. In the course of the study there was no sign of circulatory failure and the respiration was controlled artificially. However, 4 $\frac{1}{2}$ hours after the injection the patient died due to acute occlusion of the airways.

Estimation of radioactive and cold lactate, pyruvate and glucose in serum: Lactate and pyruvate in

blood was estimated by enzymatic methods. ^1C activity in lactate and glucose was estimated in serum samples after precipitation of proteins with ethanol. Supernatants were subjected to thin layer chromatography on silica gel (Kieselgel G, Merck, Darmstadt, Germany). The elution mixture was n-propanol/ethyl acetate/water 55/35/10 by vol. In this system, glucose had an R_f of approximately 0.40 and the R_f value of lactate was 0.15.

Estimation of total CO_2 and ^1C CO_2 : Artificial respiration by a respiratory pump was instituted at the age of 4 days due to respiratory failure. During the *in vivo* study with lactate 2^1C , expiratory air was collected directly from the outlet of the expiratory pump into rubber bags for periods of 5 min prior to the injection and after 1, 2, 3 and 4 hours. The CO_2 in the collected air was absorbed in a 5 N NaOH solution. CO_2 corresponding to 12 sec of respiration was quantitatively transferred by diffusion into 1.5 ml of Hyamine hydroxide 10-X (Packard) in a counting vial and the radioactivity was determined after addition of the liquid scintillator. The total CO_2 in the samples was determined as described by Stokke et al. (30).

Urine analyses: Urinary lactate was determined in a Perkin Elmer gas chromatograph model 800 at 114°C after extraction with diethyl ether and methylation with diazomethane liberated from N-nitrosomethyl urea. The stainless steel column ($6 \times 1/8$) was filled with 8% of butanediol succinate on Chromosorb W (80-100 mesh). Carrier gas was N_2 with a flow of 25 ml/min. Malonic acid dimethyl ester was used as an internal standard.

In vitro studies

In the patient as well as in a control infant, a partial autopsy was performed half an hour after their death. The control patient, suffering from multiple malformations, died at the age of 8 days. The tissues were chilled to 0°C or frozen at -20°C immediately after the removal.

Table 4 *The metabolism of lactate and pyruvate in liver and muscle slices*

Tissue slices (300–500 mg w w) were incubated in Ringer phosphate saline solution at pH 7.4 for 1 hour at 37°C. The final volume was 3.5 ml. The values are mean of two parallels. The content of liver glycogen at the start of the experiment was 1.6 g/100 g in tissue from the patient and 5.6 in the control tissue.

Substrate (μ moles)	Liver slices						Muscle slices	
	CO ₂ formed (nmoles/g w w)		Incorporated into glycogen (nmoles/g w w)		Glycogen content at the end of incubation (g/100 g w w)		CO formed (nmoles/g w w)	
	Pat	Contr	Pat	Contr	Pat	Contr	Pat	Contr
Lactate 1 C (10)	2 116	760	7	33	0.5	1.5	3 430	637
Pyruvate 1 C (10)	4 940	1 460	12	63	0.6	—	5 740	1 880

the relative contribution of the oxidation to the total lactate metabolism. From the values obtained for radioactive CO₂ exhalation when steady state condition was reached (Table 2) from the specific activity of lactate in the body and from the metabolic turnover rate for lactate it can be calculated that the oxidation to CO₂ accounts for about 60% of total lactate removed.

In vitro experiments

Tissue slices Table 4 shows that when liver slices obtained at partial autopsy were incubated with pyruvate and lactate significant amounts of ¹⁴CO were produced. The ¹⁴CO

formation was about twice as great with pyruvate as with lactate. With both substrates however the ¹⁴CO production was significantly higher in the slices from the patient than from the control. Since lactate was equally efficient as substrate in the patient as in the control a defect in LDH was apparently not present. Only small amounts of the radioactivity was recovered in the liver glycogen at the end of the incubation, about 5 times more in the slices from the control than from the patient. In the liver slices from both infants the glycogen content was considerably reduced during the incubation showing that no net gluconeogenesis had occurred. Thus as expected from the incubation conditions chosen (11), glycolysis and oxidation dominated over gluconeogenesis in the experiments with liver slices. Muscle slice from the patient and the control showed a pattern of oxidation similar to liver slices when incubated with radioactive lactate and pyruvate (Table 4).

Table 5 *Oxidation of pyruvate by liver mitochondria*

Liver mitochondria were incubated in Warburg flasks at 33°C pH 7.3 for 20 min. The temperature equilibrium period was 8 min. The main chamber contained mitochondria (7.3 mg of protein) from the patient, 11.4 mg from the control, ADP (3.3 mM), potassium phosphate (10 mM), MgCl₂ (3.3 mM), TES buffer pH 7.3 (10 mM) and KCl (10.15 M). The volume was 3 ml. The experiment was started by the addition of substrate, hexokinase (1.5 mg) and glucose (100 μ moles).

Substrate (μ moles)	Oxygen uptake (μ atoms O mg of protein/ 10 min)	
	Pat	Contr
Pyruvate (10)		
malate (10)	490	840
Pyruvate (10)	510	570
Succinate (10)	1 060	310
Caprinyl-carnitine (1)	1 060	350

Liver mitochondria The respiration rate in isolated liver mitochondria with pyruvate as substrate is shown in Table 5. As reference substrates were used succinate and caprinyl carnitine. In the control mitochondria the oxygen uptake was about half of that in mitochondria from the patient. In the patient the respiratory rate with succinate was in the same range as previously found with human liver mitochondria (14). Pyruvate (with or without malate) gave a respiratory rate which was only half the value obtained with the reference sub-

about 12% of the injected radioactivity was expired as CO₂ during the *in vivo* study

The radioactivity incorporated into glucose and liver glycogen was examined. We were unable to demonstrate significant radioactivity present in the glucose, all being found as lactate and pyruvate. In the tissues removed shortly after death the amount of radioactivity in liver glycogen corresponded to only about 3% of the dose injected 4 1/2 hours previously.

Table 3 shows the distribution of total radioactivity among various organs after death. No excess activity was found in the liver and kidney, which normally have greatest capacity for gluconeogenesis, showing that no metabolite has accumulated. It appeared to be an even organ distribution of radioactivity with an average of about 2 500 cpm/g w.w. Since the body weight was 3 kg, about 7.5×10^6 cpm of the injected radioactivity was retained, i.e. 80% of the dose. An elimination of 20% thus calculated compares well with the 13% measured (see above).

According to the estimated rate of serum lactate removal ($T/2 = 3$ hours), about 60% of the dose should have been metabolized during 4 hours. Since the oxidation, gluconeogenesis and urinary excretion can account for only 13%, the major part of the blood decay must therefore be due to a continuous diffusion of lactate into other body compartments. By calculation of the relative specific activity of respiratory CO₂ and serum lactate (Fig. 2) it was revealed that an equilibrium was not reached till 3 hours after the injection. A delay due to the mixing of ¹⁴CO₂ formed with the body pool of CO₂/HCO₃⁻ can account for maximally 20 min of these 3 hours. An equilibration period of 2 1/2–3 hours does not appear to be strikingly long when compared with the equilibration time of about 2 hours found in adults with normal blood lactate (20). A normal permeability barrier for lactate in the cell membrane has in fact been demonstrated (28) in contrast to earlier concepts based on experiments with erythrocytes (15).

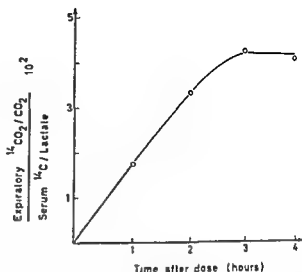


Fig. 2 The ratio between the specific ¹⁴C activity of respiratory CO₂ and serum lactate during the *in vivo* study.

In accord with the above interpretation the decrease in blood radioactivity (Fig. 1) mainly reflects the diffusion rate of lactate. Also the metabolic removal of lactate can be estimated roughly from the *in vivo* study. Lactate is assumed to equilibrate with total body water, i.e. in our patient about 2 100 ml representing 70% of the body weight. Assuming an immediate and complete mixing of the injected radioactivity, a serum radioactivity of 440 cpm/100 μ l would be expected. Using this value and the serum radioactivity after equilibration was reached (3 and 4 hours after dose) the decay curve due to metabolism can be drawn (stippled curve Fig. 1). This gives a rate constant for the metabolism of lactate of about 0.058 per hour ($T/2 = 12$ hours). Under steady state conditions therefore about 18% of the radioactive lactate would have been removed in 4 1/2 hours which compares well with the 13% measured (see above). With a body pool of lactate of 2 300 mg (distribution volume 2 100 ml, serum concentration 108 mg/100 ml) the metabolic turnover of lactate is calculated to 44 mg/kg body weight/hour. In normal adults Kreisberg et al. (20) found a mean turnover rate of about 81 mg/kg body weight/hour (range 62–96).

Finally it appeared of interest to evaluate

Table 4 The metabolism of lactate and pyruvate in liver and muscle slices

Tissue slices (300-500 mg w w) were incubated in Ringer phosphate-saline solution at pH 7.4 for 1 hour at 37°C. The final volume was 3.5 ml. The values are mean of two parallels. The content of liver glycogen at the start of the experiment was 1.6 g/100 g in tissue from the patient and 5.1 in the control tissue.

Substrate (μ moles)	Liver slices				Muscle slices			
	CO ₂ formed (nmoles/g w w)		Incorporated into glycogen (nmoles/g w w)		Glycogen content at the end of incubation (g/100 g w w)		CO ₂ formed (nmoles/g w w)	
	Pat	Contr	Pat	Contr	Pat	Contr	Pat	Contr
Lactate (C-10)	2 116	760	7	33	0.5	5.5	3 430	657
Pyruvate (C-10)	4 940	1 460	12	63	0.6	—	5 740	1 880

the relative contribution of the oxidation to the total lactate metabolism. From the values obtained for radioactive CO₂ expiration when steady state condition was reached (Table 2) from the specific activity of lactate in the body and from the metabolic turnover rate for lactate it can be calculated that the oxidation to CO₂ accounts for about 60% of total lactate removed.

In vitro experiments

Tissue slices Table 4 shows that when liver slices obtained at partial autopsy were incubated with pyruvate and lactate significant amounts of ¹⁴CO₂ were produced. The ¹⁴CO

formation was about twice as great with pyruvate as with lactate. With both substrates however the ¹⁴CO₂ production was significantly higher in the slices from the patient than from the control. Since lactate was equally efficient as substrate in the patient as in the control a defect in LDH was apparently not present. Only small amounts of the radioactivity was recovered in the liver glycogen at the end of the incubation about 5 times more in the slices from the control than from the patient. In the liver slices from both infants the glycogen content was considerably reduced during the incubation showing that no net gluconeogenesis had occurred. Thus as expected from the incubation conditions chosen (11) glycolysis and oxidation dominated over gluconeogenesis in the experiments with liver slices. Muscle slice from the patient and the control showed a pattern of oxidation similar to liver slices when incubated with radioactive lactate and pyruvate (Table 4).

Liver mitochondria The respiration rate in isolated liver mitochondria with pyruvate as substrate is shown in Table 5. As reference substrates were used succinate and capryl carnitine. In the control mitochondria the oxygen uptake was about half of that in mitochondria from the patient. In the patient the respiratory rate with succinate was in the same range as previously found with human liver mitochondria (14). Pyruvate (with or without malate) gave a respiratory rate which was only half the value obtained with the reference sub-

Table 5 Oxidation of pyruvate by liver mitochondria

Liver mitochondria were incubated in Warburg flasks at 33°C pH 7.3 for 20 min. The temperature equilibrium period was 8 min. The main chamber contained mitochondria (7.3 mg of protein from the patient, 11.4 mg from the control), ADP (3.5 mM), potassium phosphate (10 mM), MgCl₂ (1.3 mM), TES-buffer pH 7.3 (10 mM) and KCl (0.15 M). The volume was 3 ml. The experiment was started by the addition of substrate, hexokinase (1.5 mg) and glucose (100 μ moles).

Substrate (μ moles)	Oxygen uptake (μ moles O ₂ of protein/20 min)	
	Pat	Contr
Pyruvate (10)		
malate (10)	490	840
Pyruvate (10)	510	570
Succinate (70)	1 060	570
Capryl-carnitine (7)	1 060	550

about 12% of the injected radioactivity was expired as CO_2 during the *in vivo* study

The radioactivity incorporated into glucose and liver glycogen was examined. We were unable to demonstrate significant radioactivity present in the glucose, all being found as lactate and pyruvate. In the tissues removed shortly after death, the amount of radioactivity in liver glycogen corresponded to only about 0.3% of the dose injected 4 1/2 hours previously.

Table 3 shows the distribution of total radioactivity among various organs after death. No excess activity was found in the liver and kidney which normally have greatest capacity for gluconeogenesis, showing that no metabolite has accumulated. It appeared to be an even organ distribution of radioactivity with an average of about 2500 cpm/g ww. Since the body weight was 3 kg, about 7.5×10^6 cpm of the injected radioactivity was retained, i.e. 80% of the dose. An elimination of 20% thus calculated compares well with the 13% measured (see above).

According to the estimated rate of serum lactate removal ($T/2 \approx 3$ hours), about 60% of the dose should have been metabolized during 4 hours. Since the oxidation, gluconeogenesis and urinary excretion can account for only 13%, the major part of the blood decay must therefore be due to a continuous diffusion of lactate into other body compartments. By calculation of the relative specific activity of respiratory CO_2 and serum lactate (Fig. 2) it was revealed that an equilibrium was not reached till 3 hours after the injection. A delay due to the mixing of ^{14}CO formed with the body pool of CO/HCO_3^- can account for maximally 20 min of these 3 hours. An equilibration period of 2 1/2–3 hours does not appear to be strikingly long when compared with the equilibration time of about 2 hours found in adults with normal blood lactate (20). A normal permeability barrier for lactate in the cell membrane has in fact been demonstrated (28) in contrast to earlier concepts based on experiments with erythrocytes (15).

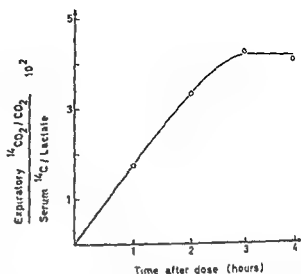


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creased production has also been proposed by Erickson (8) in another patient on the basis of lactate determinations in both venous and arterial blood after muscular exercise. Israels et al (17) and Scharer et al (29) on the other hand demonstrated an increased urinary excretion of α -ketoglutarate in the former study upon administration of glutamic acid. These findings were interpreted to indicate a defect in the citric acid cycle resulting in a decreased oxidation of lactate (12, 17, 29). A third possibility appears to us to be a block in the gluconeogenesis. That an inhibition of this pathway in fact leads to lactate accumulation is shown in animal experiments using tryptophan as an inhibitor of the gluconeogenesis (26).

Our patient had been in steady state regarding lactate metabolism for several days before the *in vivo* study. The removal of lactate from the body must therefore equal the formation and the turnover rate was estimated to 44 mg/kg/hour. This is lower than that recently found in normal adults (62–96 mg/kg/hour) (20). This allows us to exclude an abnormally high production of lactate as the cause of the hyperlactacidemia. Consequently a reduced capacity of lactate removal must exist. Lange Andersen et al (2) and Knüttgen (19) showed that after exercise the blood lactate in adults decreases exponentially with a $T/2$ of 25 min. The rate of lactate removal in our patient was estimated to equal a halftime of 12 hours. If $T/2$ had been 25 min instead of 12 hours in our patient the disappearance rate would have been 1 303 mg/kg/hour (calculated from a pool of 2 300 mg) as compared with the 44 mg/kg/hour actually found. In normal children (12) and adults (6) infusions of lactate without raising serum lactate above normal have shown a removal capacity of 150–210 mg/kg/hour which is considerably above the normal demand. When compared with previous results therefore the present study clearly shows an abnormally low capacity for lactate removal in our patient.

As discussed above one possible cause of the delayed removal of lactate might be a re-

duced capacity for its oxidation. The *in vivo* study showed that oxidation accounted for about 60% of the estimated lactate removal. In comparison Kreisberg et al (20) in adults could account for 11% of the lactate removal by oxidation. From the present *in vivo* study it may be concluded that oxidation contributes relatively much to the lactate removal in our patient. In addition the *in vitro* experiments with tissue slices as well as mitochondria did not reveal an insufficient capacity for the oxidation of lactate/pyruvate.

The other possible way of lactate removal is by gluconeogenesis. Since the oxidative pathway appeared to function normally one will *a priori* suspect a block in the gluconeogenic pathway. Several of the present observations support this concept: 1) A very low glycogen content was found both in liver and muscle. 2) Only trace amounts of radioactive glycogen in liver were found 4 1/2 hours after the injection of ^{14}C lactate. 3) No measurable amount of radioactivity could be traced in blood glucose during 4 1/2 hours after the injection of ^{14}C lactate. In contrast de Meutter & Shreeve (22) found that 10–15% of ^{14}C lactate when injected in humans was recovered in the extracellular glucose during the following 2 hours. Kreisberg et al (2) showed that gluconeogenesis accounted for at least 21% of total lactate removal in normal adults. 4) The ratio between the specific radioactivity of respiratory CO_2 and blood lactate upon ^{14}C lactate injection reached a steady level in 3 hours (Fig. 2) suggesting that a recycling of radioactivity from glucose did not occur. Kreisberg et al (20) found in adults with normal gluconeogenesis a steady increase in this ratio for more than 4 hours.

Since 15 to 20% of the blood glucose normally is derived from lactate (20, 27) hypoglycemia might be expected when a defect in the gluconeogenesis is present. However our patient received almost continuously parenteral nutrition with glucose-containing solutions from the second day of life making hypoglycemia unlikely to develop. Based on the above

strates in mitochondria from the patient whereas pyruvate + malate gave a maximal respiratory rate in the control. Since pyruvate was oxidized at the same rate even without the addition of malate as sparker, a deficiency of pyruvate carboxylase was apparently not present in the patient.

LDH-isoenzymes Very high serum levels of lactate dehydrogenase (LDH) were found in our patient (21). Moreover, the LDH isoenzyme pattern in serum appeared abnormal. The level of LDH₁ was high when compared with values from normal children aged 4–5 years (13). Further, this fraction was not detected in the control patient (Table 6).

The similarity of the patterns found in serum and liver suggests that the elevated LDH levels in serum is mainly caused by a release from the liver. This conclusion is consistent with other signs of liver damage (21). LDH₅ could not be detected in any of the tissues from the patient. The patterns in the tissues from the control appeared more normal (31, 32). The lack of LDH₁ is probably an unspecific sign since a shift in the LDH isoen-

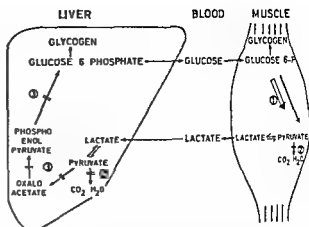


Fig. 3 Schematic illustration of lactate metabolism with indication of possible defective pathways in congenital lactic acidosis: 1 increased glycolysis; 2 decreased oxidation; 3 decreased gluconeogenesis.

zymes towards a relative decrease of LDH₅ is regularly seen in fetal tissues (7, 18) on denervation (5) and in different neuromuscular diseases (31).

Glycogen content Table 7 shows that the glycogen was reduced in the liver and not measurable in the muscle of the patient. In the control the glycogen content of the liver was in the lower normal range, whereas in muscle it was somewhat decreased as compared to that found in biopsy material from normal adults (16). The low values in the control may be ascribed to agonal changes.

Table 6 Isoenzymes of lactate dehydrogenase

The activity of each LDH fraction is expressed as a percentage of the total activity as estimated by scanning electropherograms in agarose gel. The values of the control patient are given in parentheses.

	LDH fraction				
	1	2	3	4	5
Serum	28 (30)	28 (40)	33 (30)	11 (0)	0 (0)
Muscle (ileo psoas)	25 (13)	47 (14)	28 (23)	0 (30)	0 (30)
Liver	13 (4)	23 (7)	25 (2)	39 (43)	0 (43)
Kidney	48	35	17	0	0

Table 7 Glycogen content of liver and muscle

The values (mean of two parallels) are g/100 g tissue w/w.

	Liver	Muscle
Patient	1.6	<0.01
Control	5.6	0.5

DISCUSSION

The present patient like several previously reported (4, 8, 10, 12, 17, 24, 25, 29, 33) demonstrated hyperlactacidemia of unknown reason as a cardinal sign. The existence of a metabolic error some place in the Cori cycle (Fig. 3) or in the oxidative pathway may be anticipated. The accumulation of lactate could be due to a specific enzyme defect but could also be secondary to a toxic effect of an accumulated metabolite in analogy with the accumulation of blood glycine in methylmalonic acidemia (9). Worsley et al (33) on the basis of erythrocyte experiments suggested that an increased lactate production may be the cause of the hyperlactacidemia of their patient. An in-

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findings therefore, the conclusion seems warranted that a block in the gluconeogenesis was present in our patient

Previous studies in children with hyperlactacidemia have suggested a functioning gluconeogenesis. Thus Erickson (8) found a drop in the blood lactate level across the liver in 2 cases. Haworth et al (12) observed an increase in the blood glucose levels during infusion of lactate to patients with hyperlactacidemia. The discrepancy between these results and the present findings suggests that congenital lactic acidosis might be a heterogeneous group of diseases.

SUMMARY

Biochemical studies *in vivo* and *in vitro* were performed in a newborn child suffering from congenital lactic acidosis. A block in the oxidation of lactate as well as an abnormally high rate of glycolysis have previously been suggested to be of etiological significance. In the present studies direct evidence has been obtained for a decreased capacity for gluconeogenesis as the cause of the lactate accumulation. In addition the rate of glycolysis was not increased and no block in the oxidation of lactate was present.

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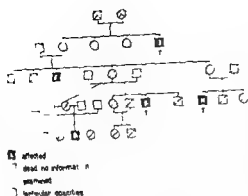


Fig 1 Pedigree of the family. Within each generation individual members are numbered from left to right. The proband is marked by an arrow.

II 5 (V H A) male infant born in 1914. He was delivered at home 3 weeks prematurely. Because of poor weight gain he was admitted to a pediatric ward when he was 2½ months old. According to the clinical record his head was enlarged and he was unable to follow objects with his eyes. Bilateral cataract was found. He was hypotonic and showed signs of mild rickets. The presence of proteinuria was also demonstrated (3 g per liter). At the age of 9 months he was completely blind and was transferred to an infant home where he soon died from pneumonia.

2 Possible cases of the Lowe syndrome

III 1 male infant born in 1925 died at the age of 9 months presumably from an infectious disease. Ocular abnormalities were not observed.

III 2 male infant born in 1926 died shortly after birth. Information about the eyes is not available.

V 1 male infant (1964) stillborn macerated. Information about the eyes is not available.

3 Carriers

II 3 born in 1945 healthy. On ophthalmologic examination the visual acuity in both eyes was 6/6+ (0.50 c) 90. In both lenses were seen peripheral coerulea elements as well as delicate opacities at the posterior pole resembling a congenital posterior polar cataract.

III 3 born in 1958 healthy. Ophthalmologic examination showed visual acuity 6/6+0.50 sph. In both lenses a delicate punctate cataract was confined largely to the embryonic core.

III 7 born in 1931 healthy. In 1959 she was examined by Seedorff (7) and by Terslev (9) who found punctate opacities in both lenses. On the present ophthalmologic examination visual acuity was 6/6+1.00 sph in both eyes. Both lenses showed incipient cataract. Minute discrete opacities were scattered throughout the lens somewhat larger rounded opacities were located at the periphery of the lens.

II 2 born in 1903 died 54 years old from cancer

of the stomach. At the age of 45 years her vision gradually deteriorated and 3 and 4 years later she underwent operation for bilateral cataract.

II 2 born in 1881. No relevant information is available.

4 Possible carriers

The following female members of the family have a 50% risk of being carriers.

II 3 born in 1908 suffers from bilateral cataract but is still able to read and has not been operated upon.

II 4 died as an infant.

II 6 born in 1948. Visus 6/6 in both eyes. In the left lens a few coerulea elements were seen temporally. Except for this the eyes were normal.

IV 12 born in 1961. Visus 6/6 in both eyes. Ophthalmoscopy normal. No signs of incipient cataract.

V 2 V 4 and V 5 likewise had normal eyes.

Three fathers of affected children were also examined. All had normal eyes without evidence of cataract. The proband's father was not examined.

AMINO ACID STUDIES

Urine from three male members (II 1, III 8 and IV 11) as well as from four female members of the family (III 7, IV 3, IV 6 and IV 12) were examined for amino acids by means of paper chromatography. In all cases normal rates of excretion were observed.

Xg BLOOD GROUP STUDIES

Seven members of the family including the proband were examined for Xg blood group antigens. Unfortunately all of these proved to be group Xg (a+). Hence no information about the relative locations of the Lowe gene and the Xg gene on the X chromosome was obtained.

DISCUSSION

All affected boys in this family showed the features typical of the oculo-cerebro-renal syndrome. Transmission was sexlinked, the complete syndrome occurring in boys only. However, Scholten (6) and Svore et al (8) have described sporadic cases in girls. Thus Svore et al reported a girl with bilateral congenital

blindness performed by Ruth Sanger, Medical Research Council Blood Group Unit, Lister Institute, London.

THE OCULO CEREBRO RENAL SYNDROME OF LOWE IN FOUR GENERATIONS OF ONE FAMILY

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Following the original description in 1952, by Lowe and associates (4) a number of reports on the oculo cerebro-renal syndrome have appeared and in 1968 Abassi et al (1) published an extensive review of its clinical aspects, adding several cases of their own. The Lowe syndrome is a congenital disease in boys characterized by ocular abnormalities, including cataract and/or glaucoma, severe psychomotor retardation, muscular hypotonia, and renal manifestations comprising renal tubular acidosis with rickets, aminoaciduria, and proteinuria.

Whereas the clinical features are thus fairly conspicuous, the genetic aspects of this syndrome remain largely unelucidated. We have had the opportunity to investigate a family in which the Lowe syndrome has been transmitted through four generations. This report presents the information gathered with particular reference to the genetic analysis.

DESCRIPTION OF THE FAMILY

Part of the pedigree of this Danish family comprising the four affected generations are shown in Fig. 1. Results of examinations of individual family members are presented in the following:

1. *Proven or probable cases of the Lowe syndrome*
IV 3 (A C) the proband male infant born in May 1967. Immediately following delivery cloudiness of the infant's pupils was noted as were marked muscular hypotonia and retention of the testes. Ophthalmologic examination revealed bilateral cataract. At the age of 3½ months elevated intraocular pressures were recorded, the corneas being cloudy and oedematous.

Several goniotomies were performed but deterioration of the boy's general condition precluded a proper therapeutic regimen and amaurosis supervened. His psychomotor development has been severely retarded and at 20 months of age his developmental age was estimated to be about 3 months and further progress has not been achieved. Renal tubular acidosis required treatment from the age of 4 months and was followed shortly by the appearance of rachitic bone changes. At the age of 7 months the patient was treated surgically for a left ureteral stricture. Intermittent glucosuria was noted through the first 3 months of life only whereas mild proteinuria has persisted. A selective amino-aciduria has been a constant finding. Serum creatinine and urea concentrations have remained within normal limits. A chromosomal analysis was normal. Further details of the findings in this patient are reported elsewhere (3).

IV 8 (C O K) boy born in 1961 delivered by Caesarean section because of fetal bradycardia. He was asphyxiated at birth and was given oxygen. Shortly following delivery bilateral buphthalmos was diagnosed, the corneas being enlarged and cloudy. Bilateral cataract was also present. The infant thrived poorly and died 4 weeks old. At autopsy a large hemangioma of the liver and partial atelectasis of the right lung were the only abnormal findings. The eyes were not examined.

IV 10 (B C) boy born in 1957. Glaucoma and cataract were present at birth. Psychomotor development was retarded and hearing impaired. Additional findings included amino-aciduria, proteinuria and mild metabolic acidosis. He died from aseptic meningitis-encephalitis at age of 1½ years. Further details of this case have been reported by Seedorff (7) and by Terlevik (9).

III 3 (V C) boy born in 1927. According to information provided by his father the infant was born at home blind with enlarged eye balls and later developed rickets. Shown a photograph of the proband the father confirmed a close resemblance. The boy died from pneumonia at the age of 8 months without being admitted to hospital.

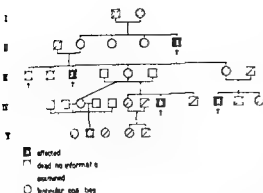


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II 3 born in 1908 suffers from bilateral cataract but is still able to read and has not been operated upon.

II 4 died as an infant.

II 6 born in 1948. Visus 6/6 in both eyes. In the left lens a few cornea elements were seen temporally. Except for this the eyes were normal.

II 12 born in 1961. Visus 6/6 in both eyes. Ophthalmoscopy normal. No signs of incipient cataract.

V 2 V 4 and V 5 likewise had normal eyes.

Three fathers of affected children were also examined. All had normal eyes without evidence of cataract. The proband's father was not examined.

AMINO ACID STUDIES

Urine from three male members (II 1, III 8 and IV 11) as well as from four female members of the family (III 7, IV 3, IV 6 and IV 12) were examined for amino acids by means of paper chromatography. In all cases normal rates of excretion were observed.

Xg BLOOD GROUP STUDIES

Seven members of the family including the proband were examined for Xg blood group antigens. Unfortunately all of these proved to be group Xg (a+). Hence no information about the relative locations of the Lowe gene and the Xg gene on the X chromosome was obtained.

DISCUSSION

All affected boys in this family showed the features typical of the oculo-cerebro-renal syndrome. Transmission was sexlinked, the complete syndrome occurring in boys only. However Scholten (6) and Svorc et al (8) have described sporadic cases in girls. Thus Svorc et al reported a girl with bilateral congenital

Kindly performed by Ruth Sanger, Medical Research Council Blood Group Unit, Lister Institute, London.

THE OCULO CEREBRO RENAL SYNDROME OF LOWE IN FOUR GENERATIONS OF ONE FAMILY

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Following the original description in 1952 by Lowe and associates (4), a number of reports on the oculo-cerebro renal syndrome have appeared, and in 1968 Abassi et al (1) published an extensive review of its clinical aspects, adding several cases of their own. The Lowe syndrome is a congenital disease in boys characterized by ocular abnormalities, including cataract and/or glaucoma, severe psychomotor retardation, muscular hypotonia and renal manifestations comprising renal tubular acidosis with rickets, aminoaciduria, and proteinuria.

Whereas the clinical features are thus fairly conspicuous, the genetic aspects of this syndrome remain largely unelucidated. We have had the opportunity to investigate a family in which the Lowe syndrome has been transmitted through four generations. This report presents the information gathered with particular reference to the genetic analysis.

DESCRIPTION OF THE FAMILY

Part of the pedigree of this Danish family comprising the four affected generations are shown in Fig 1. Results of examinations of individual family members are presented in the following.

I Proven or probable cases of the Lowe syndrome

V 3 (A C) the proband male infant born in May 1967. Immediately following delivery cloudiness of the infant's pupils was noted as were marked muscular hypotonia and retention of the testes. Ophthalmologic examination revealed bilateral cataract. At the age of 3½ months elevated intraocular pressures were recorded, the corneas being cloudy and oedematous.

Several goniotomies were performed but deterioration of the boy's general condition precluded a proper therapeutic regimen and amaurosis supervened. His psychomotor development has been severely retarded and at 20 months of age his developmental age was estimated to be about 3 months and further progress has not been achieved. Renal tubular acidosis required treatment from the age of 4 months and was followed shortly by the appearance of rachitic bone changes. At the age of 7 months the patient was treated surgically for a left ureteral stricture. Intermitting glucosuria was noted through the first 3 months of life only whereas mild proteinuria has persisted. Selective amino-aciduria has been a constant finding. Serum creatinine and urea concentrations have remained within normal limits. A chromosomal analysis was normal. Further details of the findings in this patient are reported elsewhere (3).

II 8 (C O K) boy born in 1961 delivered by Caesarean section because of fetal bradycardia. He was asphyxiated at birth and was given oxygen. Shortly following delivery bilateral buphthalmos was diagnosed, the corneas being enlarged and cloudy. Bilateral cataract was also present. The infant thrived poorly and died 4 weeks old. At autopsy a large hematoma of the liver and partial atelectasis of the right lung were the only abnormal findings. The eyes were not examined.

IV 10 (B C) boy born in 1957. Glaucoma and cataract were present at birth. Psychomotor development was retarded and hearing impaired. Additional findings included amino aciduria, proteinuria and mild metabolic acidosis. He died from aseptic meningitis and cephalitis at age of 1½ years. Further details of this case have been reported by Seedorff (7) and by Teislev (9).

III 3 (V C) boy born in 1927. According to information provided by his father the infant was born at home blind with enlarged eye balls and later developed rickets. Shown a photograph of the proband the father confirmed a close resemblance. The boy died from pneumonia at the age of 8 months without being admitted to hospital.

DISTRIBUTION OF PULMONARY BLOOD FLOW IN CHILDREN WITH CYSTIC FIBROSIS

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Pulmonary involvement is responsible for most of the complications and mortality in cystic fibrosis. Since plugging of airways with excessive secretion of mucus is one of the main features of pulmonary pathology it might be expected that besides close clinical follow up venilitory function studies should be most appropriate in assessment of the severity of pulmonary involvement (4, 8, 9, 12, 21). Unfortunately in childhood during several critical years spirometric measurements are difficult or impossible for technical reasons. Moreover the techniques are not able to detect regional differences in lung function. Therefore other methods must have been searched for.

Lung scanning has been extensively used for the evaluation of pulmonary blood flow distribution in different clinical conditions. An attempt to use the measurement of pulmonary blood flow distribution for the assessment of disturbances primarily in ventilation was stimulated by our previous work on ventilation-perfusion relationships (13). The advantage of this method for the studies of regional pulmonary function in children with obstruction of the airways including cystic fibrosis has been documented (6, 14). The present report concerns the regional changes in distribution of pulmonary blood flow and their value for the early detection and follow up of

pulmonary function abnormalities in cystic fibrosis. Special attention was paid to repeated measurements of pulmonary blood flow distribution in the same patient.

MATERIAL AND METHODS

Thirty lung scans were obtained in 19 children below 6 years of age and in 10 older patients with cystic fibrosis (Table 1). The diagnosis was based on the clinical history and on repeated physical and radiological examinations over a long period. It was confirmed by analysis of the sweat chloride concentration using the method of electrical conductivity (3). Criteria of Shwachman & Kulczycki (17) were used to score the clinical condition of our patients. Body height and weight were expressed in percentiles (18). Chest roentgenograms were obtained by the usual technique in the upright position with the exception of children younger than 1 year of age in which the supine position was preferred. The pictures were taken in the maximal inspiration. Respiratory function studies have been performed only in 5 children (case no. 4, 15, 16, 18) because of the low age of most of our patients and they are included in the previous report (7). Arterialized capillary blood oxygen tension was measured by the microelectrode of Clark type. The acid base status of the blood was determined at 37°C by the Asrap equilibration technique (1). Regional blood flow distribution was measured by lung scanning. Macroaggregated human serum albumin (particle size 10 to 75 μ) tagged with ^{99m}Tc was injected intravenously in the same position as used for taking X-ray pictures. The isotope dose ranged between 50 and 500 mCi according to the age of the patient. The total amount of albumin did not reach 0.001 mg/kg body weight. A

cataract, mental retardation and proteinuria. A sex chromatin study and a chromosomal analysis showed female characteristics. The occurrence of the syndrome in girls must be assumed to represent manifest heterozygosity in accordance with the Lyon hypothesis.

The findings in carriers are in agreement with the rather limited evidence on record. In the present family, all carriers had lenticular opacities. However, the lenticular changes varied, coerulea elements, peripheral spokes and posterior polar cataract being found within the family. Wilson et al (10) likewise observed polar cataract in one carrier. Abassi et al (1) did not find lenticular changes in all mothers of affected children. On the contrary, in one family studied by them, the father of an affected boy had lenticular opacities and similar observations in fathers have been reported by Richards et al (5), Illig et al (2), and Wilson et al (10). However, slight lenticular changes occur frequently and may be observed in 25% of all persons from the age of 40 years. Hence the fact that lenticular opacities are found in some fathers does not necessarily mean that a particular parental configuration is required in order to produce the complete syndrome. The cataracts found in the eyes of carriers correspond well to those which may be observed in an appreciable fraction of an ophthalmologic clientele. However, in the family studied by us, the changes seem to appear early, and in one conductor (II-2), operations were required before the age of 50 years. Even though another carrier (III-7) still has a normal vision, the incipient cataract will probably soon cause visual disturbances. Although lenticular changes may not occur in all carriers, we find it advisable, prior to genetic counselling to perform an ophthalmologic examination on possible carriers of the Lowe syndrome.

SUMMARY

A Danish family with five proven and three additional possible cases of the oculo-cerebro-renal syndrome is described. All proven carriers

had cataract, but the lenticular opacities varied considerably with respect to magnitude, appearance, and location. In this family, the disease was transmitted as a sex-linked recessive trait, the complete syndrome being seen in boys only.

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Chest roentgenogram				Lung scan	
Peribronchovascular thickening		Localized density	Hyperaeration Grade	Underperfusion	
Grade	Localization			Grade	Localization
0	—	0	0	0	—
2	RUt RLt	0	1 d	3	RUp RMp
1	LLp			2	RUt
1	RUt LUt	RUt	1 d	0	—
1	RUt LUt	RUt	1 d	2	RUt
				1	RLp
	RMp RLp LLp	0	1 d	3	LUt
				2	RMp
2	RMt RLt LLp	0	2 d	2	LUt RMp
1	RUt RLp LUt	0	0	0	—
1	RUp RLp	0	0	0	—
1	RUt RMt LUt LMt	0	2 d	1	RUt
2	RUp RLp LUp LLp	0	1 d	0	—
2	RUt RMt LUt	0	1 d	1	Multipf
3	Generalized	0	2 d	3	RUp RMp LUt
				2	RUt RLp
1	RLp LLp	RLp	2 d	3	RUp RMt
1	RUt LUt	RLp	3 d	2	RLp LUp
1	RUp RLp	0	1 d	1	LUp
2	RLp LUp	RLp LUp	1 d	0	—
2	RUt RMt RLt LMt	LMp RUp	2 d	0	—
3	RMt RLt LMt LLt	RMp LUp	3 c	2	RUp LUp
3	RUt RMt RLt LUp LLt	LLp	2 d	2	RUp RMp RLp LLp
				2	RUp RMp RLp LLp
3	RMt RLt	0	3 d	3	RUt LUt
				2	RMp LUp
3	RMt RLt	0	3 d	3	RUt LUt
				2	RMp LUp
3	RMt RLt	0	3 d	3	RUt LUt
				2	RMp LUp
3	Generalized	RLp RMp LLp	2 c	3	RUt
				2	LUp LLp
3	Generalized	0	3 d	3	RUt RMp LUp
				2	LMp
3	Generalized	0	3 d	3	RUt RMp
				2	LMp
2	RUp RMp LUp LUp	RUp LLp	2 d	2	RUp RMp RLp LLp
2	RUt RLp LLp	0	1 d	0	—
2	RUp LUp	RLp	1 d	0	—
				2	RUt

series (Table 1) The most frequent incidence of local changes in perfusion was encountered in the right upper area followed by the right middle and left upper area (Table 2 and Fig. 1) The combination of partial and total involvement of several areas was usual Only in 4 children solitary total or partial underperfusion of one area appeared Marked and moderate changes in perfusion or their combination were most frequent

By dividing our patients according to the

Table 2 Regional distribution of underperfusion

Lung area	Incidence of underperfusion (%)
Right	
Upper	81
Middle	67
Lower	29
Left	
Upper	57
Middle	79
Lower	33

Table 1 Data on patients and results of studies^a

Case no	Name	Sex	Age (y mo)	Height (per centil)	Weight (per centil)	Sweat Cl (mEq/l)	Clinical score	Arterial blood			
								Po ₂ torr	Pco ₂ torr	pH	HCO ₃ (mEq/l)
1	P T	♂	3 3	70	50	96.6	85	82	31	7.362	171
2	R D	♀	5 9	50	70	102.4	84	73	39	7.386	230
3	M H	♀	11 7	40	50	114.4	78	85	36	7.406	217
			0 7	40	50	—	78	—	—	—	—
4	M V	♀	11 7	20	30	100.4	78	102	37	7.450	251
			11 8	20	30	—	78	90	14	7.455	235
5	M M	♂	1 5	90	50	103.9	77	90	11	7.419	194
6	E J	♂	4 9	10	70	81.7	76	112	28	7.470	178
7	A H	♂	3 6	10	20	128.4	73	—	31	7.440	200
8	J P	♀	4 9	10	40	104.4	68	—	—	—	—
9	P H	♂	3 10	10	3	124.4	67	103	38	7.456	250
10	T L	♂	5 0	3	20	115.4	65	70	37	7.398	220
11	P M	♂	1 1	10	10	108.9	64	70	37	7.400	223
			2 2	30	50	—	63	80	37	7.371	207
12	H P	♀	3 6	10	3	89.2	63	93	39	7.385	230
13	L B	♀	1 8	3	3	116.9	63	90	39	7.412	245
14	M S	♂	0 5	70	10	82.6	55	75	49	7.173	281
15	M H	♂	3 0	80	20	117.4	55	—	48	7.143	253
			4 7	20	30	—	49	76	51	7.155	281
			4 8	20	30	—	—	—	45	7.409	280
16	L S	♂	20 2	90	40	70.2	54	—	—	—	—
			20 3	90	40	—	54	53	41	7.432	269
			20 4	90	40	—	54	55	40	7.476	259
17	D F	♀	4 4	3	10	84.4	51	58	42	7.393	248
18	J H	♂	5 11	3	3	120.5	50	100	34	7.442	213
			6 11	5	3	—	46	70	33	7.406	203
19	K M	♀	4 5	30	3	115.4	50	80	40	7.458	270
			4 6	30	3	—	45	80	40	7.439	261
20	L M	♀	2 1	3	3	101.3	49	77	44	7.388	256
			3 8	3	3	—	52	84	34	7.399	202
21	A D	♀	1 0	10	3	91.9	48	83	29	7.448	192

^a Grade 0 = normal 1 = slight 2 = moderate 3 = marked Localization RU RM RL = right upper middle lower area LU LM LL = left upper middle lower area t = total p = partial d = diffuse c = cystoid

rectilinear scanner (Pho/Dot Nuclear Chicago) equipped with 127 hole collimator was used for the detection of the distribution of the macroaggregates. Both X ray picture and lung scan were usually taken within the shortest possible period of time in the same day.

The evaluation of regional perfusion was done in a similar way as in the chest roentgenograms. Both lungs were divided by the horizontal lines into three even areas not corresponding exactly to the lung lobes. Peribronchial thickening, hyperaeration on roentgenograms and underperfusion on lung scan was divided according to the intensity into 3 grades. The radioactivity equal to the background activity is

called underperfusion of the grade 3 in contrast to the underperfusion of the grade 0 equal to the maximal radioactivity concentration. Partial or total involvement of individual areas indicates the extent of these changes. In 8 patients repeated measurements of regional pulmonary blood flow were performed with an interval ranging from 1 week up to 1 year and 8 months.

RESULTS

Normal distribution of the pulmonary blood flow was detected only in 8 children in our

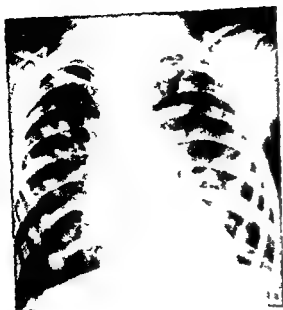
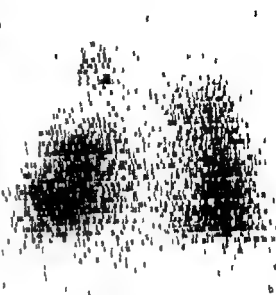


Fig 3 T L (case 10) (a) Chest roentgenogram (b) Lung scan Multiple defects in perfusion con-



trasted with the generalized image on chest roent genogram

Both normal and pathological local distribution of pulmonary blood flow was detected in children with the impairment of pulmonary gas exchange indicated by the elevation of P_{CO_2} and by the decrease in P_{O_2} values in arterialised capillary blood. Correlation between the severity of distribution abnormalities and disturbances in gas exchange was not revealed.

Radiological evidence of pulmonary involvement participates in clinical scoring. However, comparison of lung scans with chest roentgenograms alone was necessary for the estimation of the value of both techniques in patients with cystic fibrosis (Table 1). In all but one patient peribronchial thickening of different intensity was evident from the chest roentgenogram. In the majority of cases localization of radiological changes and abnormalities in the distribution of radioactivity correlated only partially. In one third of investigations marked discrepancy was found. Focal changes in density were detected on 11 roentgenograms. Their localization did not agree in 64% of investigations with the localization of perfusion inequalities. On the other hand areas of marked underper-

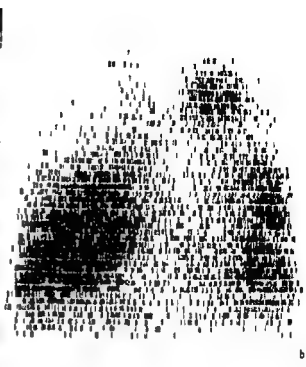
fusion were revealed by lung scanning in patients without focal changes in X-ray density (Fig 3).

Repeated scanning was performed in 8 patients. Different pictures of blood flow distribution were obtained in 5 patients (case 3, 11, 15, 18, 19). Marked changes in the distribution pattern within an interval shorter than 1 month were revealed in two of them without apparent change of the clinical condition (case 3, 11). In the third child (case 15) the spreading of distribution abnormalities after a longer interval was accompanied by the deterioration of the clinical condition. The change only in the localization of underperfused areas was combined with the lowering of clinical score in two children (case 18, 19).

Unchanged distribution was found three times (case 4, 16, 20) after an interval of 1 month or 1 year respectively. The discrepancy between stationary pattern of blood flow distribution and changing clinical picture was detected in two patients (case 16, 20). During the first measurement one of them (case 20) was in a fair clinical condition. Surprisingly lung scan showed marked disturbances in per-



Fig 1 J H (case 18) (a) Chest roentgenogram shows generalized marked peribronchial thickening and diffuse hyperinflation (b) Lung scan shows most



frequent localization of perfusion defects (see Table 1)

clinical scoring method (17) three groups became apparent. Only in 50% of the patients in good clinical condition (case 1-7) normal pulmonary blood flow distribution was revealed (Fig 2). High frequency of abnormal

ties in distribution was encountered in the group with mild clinical condition (case 8-13) and in the group with the lowest clinical score (case 14-21). Nevertheless, in the latter group normal distribution pattern was also detected

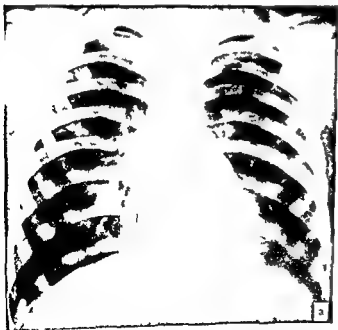


Fig 2 R D (case 2) (a) Chest roentgenogram shows moderate degree of peribronchial thickening mostly of the right side with diffuse hyperinflation



of low degree (b) Lung scan demonstrates abnormalities in blood flow distribution not predictable either from clinical or radiological examination

local increase in pulmonary vascular resistance and consequently decrease of blood flow in this region (1).

This mechanism was proved to be responsible for the perfusion abnormalities under several conditions leading to the local obstruction of the airways. In acute asthmatic attack marked local defects in perfusion were demonstrated in children with otherwise normal pulmonary blood flow distribution (10, 16). To prove that the obstruction of airways is responsible for the change on lung scan blood flow distribution was measured after partial bronchial obstruction by unilateral bronchography. Transient perfusion abnormalities were of the same character as during an asthmatic attack (16). The changes in regional perfusion following localized obstruction of the airways were not identical with the changes in regional ventilation (19). On the contrary Gyepes et al (6) were able to demonstrate the local coincidence of ventilation and perfusion defects. The existence of perfusion disturbances and their variability in our patients favours the participation of mechanism regulating perfusion according to the ventilation in pulmonary pathophysiology of cystic fibrosis.

One question remains the efficiency of this mechanism in maintaining perfect gas exchange. If the diminution of perfusion matches locally and qualitatively the decrease in ventilation no disturbance in pulmonary gas exchange would develop. However the studies of local regulation of ventilation/perfusion ratio have shown some limitations of this mechanism (15, 20). This may explain low ventilation/perfusion ratio revealed in patients with cystic fibrosis (11). Whatever is the efficiency of this mechanism local inequalities of perfusion in our patients with the obstructive lung disease must have favourable effect on gas exchange. If the lungs were not able to shift blood away from underventilated regions the incidence of abnormal values of arterial gas tension might be expected to be high.

Roentgenography has been widely used in the assessment of pulmonary involvement in

cystic fibrosis. The limitations of this method in detection of local changes in perfusion has been demonstrated by comparison with lung scanning in this study. The lack of correlation between both methods was encountered. The most frequent finding on chest roentgenograms was peribronchial thickening and diffuse hyperaeration. There is no reason to expect changes of this character to be reflected by the local abnormality of perfusion. Localized changes of X-ray density without regard of their etiology should manifest themselves as the change in perfusion. This was not the case in the majority of our patients. On the other hand even marked defects in perfusion shown by lung scanning were not revealed by roentgenography. In view of these facts there is no reason to underestimate the value of roentgenography in cystic fibrosis. Its importance is primarily in depicting anatomical pulmonary lesions and its value was documented also in our study. The main advantage of lung scanning is in the detection of regional changes in perfusion i.e. in the detection of a functional abnormality. Our study has shown that both methods are very useful but not interchangeable.

By use of criteria indicated above three clinical groups of patients were differentiated in our material. The explanation of the lack of exact correlation between the severity of the illness and incidence of the disturbance in perfusion must be searched for in the above mentioned mechanism of development of the perfusion changes. If we accept the assumption that the regional abnormalities in perfusion are secondary to the ventilation disturbances then the defects in perfusion depend solely on the magnitude and character of the airway obstruction. On the other hand the clinical picture is composed also by many other factors.

Variability in bronchial obstruction is typical for the early stage of pulmonary involvement whereas fixed changes can be interpreted as permanent disturbance of pulmonary function signaling the unfavourable progression of the disease (8). Early detection of dynamics

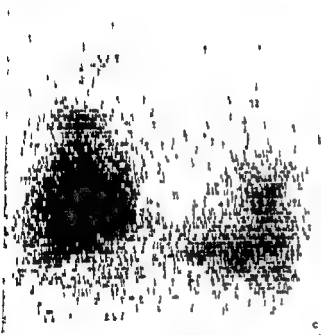


Fig 4 I S (case 16) (a) Chest roentgenogram taken at the same data as the second lung scan shows marked peribronchovascular thickening and hyperaeration with decreased density in left upper area (b) and (c) Lung scans recorded with an interval of 10 weeks show identical pattern of blood flow distribution

fusion (Fig 4 b) These changes did not improve after the intensive treatment One week later this patient was admitted to the hospital in respiratory distress due to acute respiratory infection and a small localized left sided

pneumothorax After the recovery an identical distribution pattern persisted (Fig 4 c)

DISCUSSION

Lung scanning is believed to be very useful in the diagnosis of pathologic processes involving the pulmonary vascular bed. However, no significant changes in pulmonary arteries and arterioles were found in cystic fibrosis (5). The high incidence of the abnormalities in pulmonary blood flow distribution proved in our patients must therefore be caused by another mechanism. This mechanism has not been firmly established yet. It is reasonable to assume that disturbances in the patency of the airways play an important role in evoking secondary changes in perfusion by two principal mechanisms. The obstruction of bronchi and/or bronchioles is followed by profound changes in the composition of alveolar gas, namely decrease in P_{O_2} and increase in P_{CO_2} . Increase in the airway resistance causes the enhanced respiratory fluctuations of transpulmonary pressure. During expiration, alveolar pressure distal to the partial airway obstruction increases considerably (9). Both changes in alveolar gas tension and in intrapulmonary pressure elicit

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of these functional abnormalities by repeated lung scanning may have considerable implications in the management especially in the age when lung function studies are not available. Repeated scanning can be used also as one of the indicators of the therapeutic effect. Lung scanning as a highly reproducible simple and relatively safe procedure, available in any age offers new possibilities in clinical follow up of patients with cystic fibrosis and it should be included among other criteria for the assessment of clinical condition and mainly pulmonary involvement in cystic fibrosis.

SUMMARY

Marked abnormalities of pulmonary blood flow distribution were detected in 13 out of 21 children with cystic fibrosis using lung scanning. Underperfusion of different grade was localized most frequently in the right upper area followed by the right middle and left upper areas. Correlation between clinical condition or impairment of gas exchange and degree of perfusion disturbances was lacking. Abnormal chest roentgenograms were encountered more frequently than the definite disturbances in the distribution of perfusion. On the other hand even profound defects of perfusion were not revealed by chest roentgenography. Repeated lung scanning might be helpful in the evaluation of the dynamics of pulmonary involvement in cystic fibrosis.

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Table 1 Model employed in first analysis

The model fitted was

$$\frac{1}{2} \log p(1-p) = \frac{b}{a}$$

$$b_{sch1} + b_{sch2} + b_{sch3} + b_{sch4} + b_{sch5}$$

$$b_{sc6} + b_{sc7} + b_{sc8}$$

$$b_{sch1} + b_{sch2} + b_{sch3} + b_{sch4}$$

where a is age in half years
representing the overall mean + the effects of
school 5 and social class 3
deviations due to other social classes
deviations due to unemployment parental
separation paternal disease
deviations due to other schools

girls attain menarche are estimated as 10.99 and 15.87 years (Fig 1).

If the school variables are assumed to have no effect a significantly worse fit (measured by χ^2 , $p < 0.05$) is obtained than in the complete model. On the other hand omitting the social variables does not make a significant difference to the γ testing: goodness of fit. Thus the results show no social class effect but there does appear to be some difference between schools.

These findings are somewhat surprising. The differences are illustrated in Table 2: fitting out the main effects model fitted to all the data. In the right hand margin appear the median values for each school if social effects are taken as zero and at the lower margin appear the medians for each social category ignoring differences between schools. The junior school (school 5 the smallest sample) is perhaps by chance notably late but it does not account for the differences between the schools for if it is excluded differences between schools remain significant. Of the remainder the grammar school gives the earliest mean age, the

Catholic school the latest mean age and the comprehensive and secondary modern are very similar to each other. Apart from the fact that these differences are not explainable by the social categories the analysis so far suggests nothing as to their cause.

The overall values for the social categories shown at the foot of the table which ignore the differences between schools though not significant are of interest in that they include data relating menarcheal age to family difficulties. Social classes 2 to 5 are remarkably consistent, and the median for girls with parents separated is very similar to these. Girls with fathers unemployed appear to have the latest menarcheal age and where the father is dead the median is early. These suggestions obviously call for further enquiry.

Second analysis To investigate the effects of the two further factors family size and family position required a larger model and for computing reasons it was necessary to restrict the number of other variables. Of the social variables only social classes 1 to 5 were considered in this analysis and the 109 children whose father was unemployed or dead or whose parents were separated were omitted. At the same time social classes 1 and 2 were combined.

The complete model in this analysis included variables of family order representing combinations of family size and position in sibship e.g. first of two siblings, second of three. After the complete model was fitted the null hypotheses were tested that the school, parents, social class and family order alone and in pairs had no effect. The results obtained are shown in Table 3.

Again the results show no indication of a

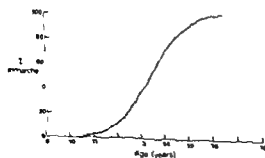


Fig 1 Proportion by age of girls who have attained menarche in South Shields.

AGE AT MENARCHE PHYSIQUE AND ENVIRONMENT IN INDUSTRIAL NORTH EAST ENGLAND

D F ROBERTS L M ROZNER and A V SWAN¹

*From the Laboratory of Human Genetics University of Newcastle upon Tyne
and the Medical Research Council Clinical Research Centre
Division of Computing and Statistics London England*

In the last 20 years have appeared several studies of current age at menarche in Britain, which show a pronounced secular trend to earlier maturation and little in the way of variation from one region to another. The majority of these surveys however relate to the southern part of England and there has been no investigation of a population in the industrial north east. This study was undertaken to remedy this deficiency.

MATERIAL AND METHODS

The study was carried out in 1967 in the urban community of South Shields County Durham and surveyed girls ranging in age from 9 to 16.

For each girl was recorded the date of examination her date of birth her height weight and whether she had attained menarche. Social data were also recorded—family size as indicated by the number of sibs the girl's position in the family and the social class defined by father's occupation as in the Registrar General's classification. Other facts noted were whether she was a twin and where relevant supplementary social information—whether the family was in economic hardship and received National Assistance whether the father was unemployed or in prison or deceased and whether the parents were divorced or separated. Halfsibs stepsibs and fostersibs were all included in the measure of the family size. Two girls in the care of the Local Authority and the few coloured girls were omitted giving a total sample of 1 654 subjects from these were eliminated the twins

and fatherless children with mothers on National Assistance since they were so few leaving a final sample of 1 608. Five schools were included: 1) a comprehensive school 2) a grammar school 3) a Catholic secondary school 4) a secondary modern school and 5) a junior school. The sample thus relates to a north east English industrial urban schoolgirl population.

RESULTS

1 Menarcheal age

The analysis consisted of the following. A logit transformation $y = \frac{1}{2} \log (p/(1-p))$ was applied to the proportion p which had attained menarche at each age. This was assumed to depend on a linear function of the main effects of all the variables investigated and a multiple regression technique was used to estimate these effects (e.g. Table 1). Groups of these effects taken in turn were then assumed zero and the significance of the additional residual heterogeneity was examined.

First analysis The model was first fitted relating the logit of the proportion menstruating to variables (in the form 0.1) representing school social influences (the parents' social class paternal unemployment parental separation paternal disease) and the girl's age to the nearest $\frac{1}{2}$ year (Table 1). If all the variables but age are assumed to have no effect an overall median age at menarche of 13.43 years was obtained with a standard error of ± 0.05 years. The age limits between which 95% of

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Table 5 Main effects

Coefficient for each variable to be added to overall median and number of children on which the estimates are based

Variable	Coefficient	No of children
Schools		
1	40	343
2	53	614
3	76	197
4	0	255
5	0	92

Social class		
1 & 2	06	183
3	0	737
4	01	478
5	13	153

Family					
Size	Position	(a)	(b)		
1	1	04	12	146	146
2	1	0	0	199	398
	2	16	0	199	
3	1	4		136	
	2	43	26	143	374
4	3	34		95	
	4	7		53	
	5	37	47	131	70
	6	49		76	
5	7	44		69	
	mid	47	45	781	363
	last	55		23	

To estimate the median menarcheal age for any group of girls in this sample three coefficients must be employed one from each of the sections school social class and family table for (a) if it is ship position is required column (b) if it is not.

Physique

The inter relations of age at menarche physique and the environment in which girls develop are complex and from data that are not longitudinal and therefore cannot include.

Table 6 Second and third analyses

Median age at menarche when family size is the sole variable analysed

Family size	Median
1	13.04
2	13.14
3	13.45
4	13.68
5 or more	13.66

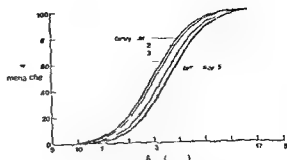


Fig. 2 Effects of family size on menarcheal age

growth velocities comprehensive analysis is not possible. As a more limited objective the South Shields data were analysed to discover which of the variables previously considered exerted any detectable influence on height weight and the ponderal index (height³/weight) calculated therefrom. This was done by obtaining the multi linear regressions of each of the three physique variables in turn on variables in the form of 1 representing the schools social class family position and menarcheal state. The age of the girls introduced a complication for there is during puberty a non linear relationship between the physique variables and age. To overcome this the data were divided into yearly age groups and the regressions performed separately within each of these groups. This procedure allows age to be considered as held constant albeit approximately and omitted from the analysis while the effects of the other variables are investigated.

In the age range 10 to 11 years there are 46 children none of whom has reached menarche. The only physique variable that shows a significant regression in this age range is the ponderal index ($p < 0.05$) for there is a tendency for the index to increase with the family order variables. The analysis was repeated with family size variables replacing the family order (combined size and position) variables again the regression of ponderal index on these is significant ($p < 0.05$). As in the menarche analysis it seems to be the size of the family rather than the position of the child in that family which influences ponderal index in this age

Table 2 Median age at menarche

First analysis Main effects model fitted to all data (ages in years)

	Social class					Father unemployed 6	Parents separated 7	Father deceased 8	Overall
	1	2	3	4	5				
School 1	12.47	13.46	13.48	13.50	13.37	13.66	13.48	12.80	13.46
2	12.29	13.28	13.30	13.32	13.19	13.48	13.30	12.62	13.36
3	12.74	13.73	13.75	13.77	13.64	13.93	13.75	13.07	13.74
4	12.43	12.42	13.44	13.46	13.33	13.62	13.44	12.76	13.43
5	13.05	14.05	14.06	14.08	13.95	14.25	14.06	13.38	14.04
Overall	12.30	13.37	13.43	13.49	13.39	13.67	13.40	12.74	

social class effect. Neither in this analysis do they show any indication of a school effect either alone or combined with social class. On the other hand they do show a significant family order effect ($p < 0.025$).

Third analysis. A reduced model in which the family order variables were replaced by variables representing family size alone made it possible to test the significance of position in sibship and family size separately and the results are shown as subdivisions of the family order χ^2 in Table 3. The family order effect is almost entirely due to the family size effect which is highly significant ($p < 0.005$) while the position in sibship does not appear to matter. These results suggest quite strongly that the significant school effect obtained in the first analysis was the result of systematic differences in family size from school to school. This is illustrated by the family sizes for each school given in Table 4: the differences between the schools are pronounced.

The coefficients of the various factors in the second and third analyses and the numbers of children on which they are based are set out in Table 5. These coefficients are to be added to the overall median to obtain that for any particular category of child e.g. girls at school 2 of social class 4 who are the second in a family of three have a median menarcheal age of $13.43 - 0.53 - 0.01 + 0.43 = 13.32$ years. Since of these the only factor found to be of significant effect was family size the medians when family size alone is analysed are set out in Table 6 and the distributions in Fig. 2.

In summary then the results show no independent effect of social class or of family position. In the South Shields data the two main factors of those analysed that influence whether a girl has attained menarche or not are her age and the size of the family from which she comes.

Table 3 Second and third analyses

 χ^2 values for the goodness of fit of the models

	χ^2	d.f.	p
Complete model	611.78	761	
Increase due to constraining hypotheses			
No family position or size effect	22.27	11	<0.025
No family position effect		4.00	7
No family size effect		18.27	4 <0.005
No social class effect	0.70	3	
No school effect	4.03	4	
No school or social class effect	4.65	7	

Table 4 Numbers of children with a given family size within schools with the mean family size

School	Family size					Mean size
	1	2	3	4	5+	
1	37	92	86	59	69	3.3 ± 0.10
2	86	228	154	70	76	2.8 ± 0.06
3	3	21	41	40	92	4.6 ± 0.15
4	16	47	57	33	102	4.4 ± 0.16
5	4	11	35	18	24	3.8 ± 0.16

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4	- 01	428			
5	- 13	153			
<i>Family</i>					
Size	Position	(a)	(b)		
1	1	- 04	- 12	146	146
2	1	0	0	199	398
	2	- 16		199	
3	1	74		136	
	2	+ 43	- 26	143	374
4	3	- 34		95	
	1	2		53	
	2 & 3	- 32	47	131	270
	4	- 43		36	
5	1	- 44		59	
	mid	47	45	281	363
	last	55		23	

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The inter relations of age at menarche physique and the environment in which girls develop are complex and from data that are not longitudinal and therefore cannot include

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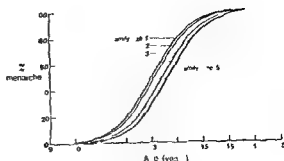


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Table 2 Median age at menarche

First analysis: MMR effects model fitted to all data (ages in years)

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	1	2	3	4	5				
School 1	12.47	13.46	13.48	13.50	13.37	13.66	13.48	12.80	13.46
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	3	34 95
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	last	- 55 23

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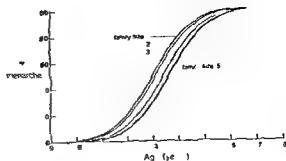


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The results over the whole age range in these children indicate that of the factors investigated only menarcheal status has a consistent effect on physique. Table 9 gives the estimated effects of menarche on height and weight for each age group and the numbers of children. The four girls in the youngest age group who are ahead of their age mates in menarche are also very much ahead of them in stature while the seven girls in the 15 to 16 age group who are lagging in maturation are also considerably shorter suggesting that it is at the extremes of maturation that its relationship with physique may be most pronounced.

DISCUSSION

Menarche

The variables examined in this survey were chosen as a result of some rather unexpected findings of an investigation amongst Swansea college girls (4) where the method of survey was by recall on a series of cross sectional samples. Besides a secular trend in menarcheal age significant effects were detected of family size and position in family but there was no effect of social class. It was obviously necessary to substantiate these findings on a sample at which the criticism that the data were open to recollection error could not be levelled and the method employed here collecting data on whether menarche had or had not been attained with subsequent logit analysis was the obvious choice.

The South Shields results do in fact provide reasonable confirmation. They endorse the absence of a social class effect on menarcheal age and are in this compatible with other recent studies in Britain (2, 3). The family size effect again confirms the Swansea findings not only in its occurrence but also in its magnitude in the present sample (family sizes 1 to 4 inclusive) averaging 0.18 years per additional sib by comparison with 0.154 ± 0.038 years per additional sib in the Swansea results. The South Shields findings however differ from the Swansea results in the absence of a significant family

position effect yet inspection of the family position coefficients in Table 5 suggests that in the largest families (of size 4 and size 5 and above) the youngest sib may indeed be accelerated by comparison with the oldest and middle sibs though this is not sufficiently marked to be distinguished from chance fluctuations in this sample. Further investigation of larger families is clearly required.

Taken overall these findings like those of the Swansea investigation suggest that environment (and in particular standards of nutrition and general care) is still an important determinant of age at menarche but that today in Britain it operates through family size and no longer through accepted socio-economic categories. The Registrar General's categories are today it seems a less effective measure than formerly of differences in housing standards, expenditure on food and other variables of potential biological relevance. To these instead family size appears to be a much more sensitive indicator, poverty that is effective in that it influences biological development should today perhaps be sought among those of large families instead of those of the lower classes. However it is possible that some other factor not considered in this study e.g. some genetic influence may be affecting families of different sizes by different amounts.

The overall median age (13.43 years) is in fact later by one third of a year than that in the last reported status quo sample from Britain (6). This delay may perhaps indicate an interruption or termination of the secular trend to earlier menarche. On the other hand it may be merely a reflection of the characteristics of this sample drawn from an urban industrial area where there is still much economic hardship. The latter interpretation is compatible with the environmental effect on maturation postulated above. Or perhaps the delay is due to a combination of both.

Physique

The number of analyses undertaken in the physique investigation colours their interpretation

Table 7 Mean ponderal indices against family size for children in the 10-11 year age group

Family size	Mean P I
1	12.38
2	12.77
3	12.77
4	13.27
5+	12.59

Table 8 Mean age by schools within the 11-12 year age group

School	No. of girls	Mean age in years
1	30	11.83
2	17	11.91
3	23	11.77
4	12	11.88
5	37	11.30

group. The mean ponderal indices are given in Table 7.

In the age range 11 to 12 years (119 children) the regressions of both height and weight are significant ($p < 0.01$) due partly to differences between schools (height $p < 0.01$, weight $p < 0.05$) and social classes (height and weight $p < 0.05$), but mainly menarcheal status (height and weight $p < 0.001$). However, this group includes 37 children from the junior school (5) whose average age and consequently their average height and weight within the one year age group is considerably less than those of the children in the other four schools (Table 8). When these children are omitted the significance of the differences between schools disappears and so does the effect of social class on height. This leaves only the regressions of height and weight on menarche and of weight on social class as significant. This last appears

to be due to the fact that in this age group the few children in social classes 1 and 2 are particularly heavy in their combined average about 25 lbs heavier than children of equivalent menarcheal state in the other social classes. The finding in this age group that menarcheal status (whether the child had reached menarche or not) has a significant effect on height and weight but not on ponderal index is surprising in view of the consistent decrease in the index associated with menarche found in the 12-15 age groups.

In the age ranges 12 to 13, 13 to 14 and 14 to 15 years with 304, 366 and 351 children respectively all the regressions are significant ($p < 0.01$) but solely on account of the effect of menarcheal state except for an isolated instance in the range 14 to 15 years. The exception in this range is the regression of height on school which is also significant ($p < 0.01$) apparently on account of the children in the secondary modern school (4) being on average 0.5 inch shorter than children in the other three schools, even after allowance for social class and menarcheal state.

In the age ranges 15 to 16 and 16 years and more with 216 and 90 children respectively the regressions of ponderal index are no longer significant and in the latter range neither is the regression of height. Those regressions which are significant in these age ranges are again solely due to the regression on menarcheal state. In these groups only seven and one girl respectively have not reached menarche so differences between those who have and those who have not would have to be very large to reach significance.

Table 9 Estimated effects of menarche on height and weight

Age group	No. of children	No. menstruating	Increase due to menarche of	
			Height (ins.)	Weight (lbs.)
11-12	82	4	6.2 ± 1.3	33.5 ± 8.6
12-13	304	76	2.1 ± 0.4	18.2 ± 2.4
13-14	366	178	2.0 ± 0.3	21.6 ± 1.9
14-15	351	285	1.6 ± 0.3	18.9 ± 2.3
15-16	216	209	3.2 ± 1.0	21.5 ± 7.5

TEST MEAL IN THE DIAGNOSIS OF MALABSORPTION IN INFANCY

Tolerance Tests Using Simultaneous Oral Administration of Glucose D Xylose Cream and Vitamin A

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In the clinical routine work up of patients with symptoms suggesting a malabsorption syndrome a series of tolerance tests is usually performed in order to obtain a pathogenetic diagnosis. Two of the major diagnostic tests are the D xylose (9) and vitamin A (1) tests. Usually glucose tolerance test is also performed (11). Cream is sometimes preferred to vitamin A as a test of fat absorption (3).

The purpose of this communication is to report studies on the diagnostic value of a test meal containing glucose D xylose cream and vitamin A in infants with various disorders causing malabsorption syndrome. Normal in infants and patients with cystic fibrosis extrahepatic biliary atresia intrahepatic cholestasis (i.e. neonatal hepatitis) and gluten induced enteropathy have been examined. The amount of the individual constituents of the meal was similar to those usually given in isolated tolerance tests. Following the combined test meal the blood concentrations of glucose D xylose triglycerides and vitamin A have been determined.

MATERIAL

No mal infants

Sixteen infants 9 boys and 7 girls with normal growth pattern were studied. There was no evidence of infection anemia or gastrointestinal disturbances.

This study has been supported by grants from the Swedish Medical Research Council (60) from Stockholm Läns Landsting and from Karolinska Institutet's reservationsslag.

Infants with malabsorption syndromes

Three infants with cystic fibrosis 3 with extrahepatic biliary atresia and 4 with intrahepatic cholestasis (neonatal hepatitis) were studied.

Four infants with gluten induced enteropathy (celiac disease) were studied before a gluten free diet was instituted. All of them responded to the gluten free diet and developed clinical symptoms upon provocation with gluten.

Clinical data are given in Table 1.

METHODS

Cholic acid 24-C was administered intramuscularly to the patients with cholestasis. The urinary and faecal isotope excretion was determined as described earlier (12).

Analytical methods are given in Table 2.

TEST-MEAL

The infants received a diet regular for their ages during the 3 days preceding the test meal. Infants younger than 2 months of age fasted 6 hours. Older infants 9 hours. A feeding tube was then put into the stomach through the nose and vitamin A palmitate was given through the feeding tube immediately followed by a solution containing glucose D xylose and cream. The amount of the four constituents administered was as follows: vitamin A palmitate (Ido-A[®] Ferrosan 100 000 IU per ml) 7 500 IU per kg body weight glucose 2 g per kg body weight (with a minimum dose of 20 g) D xylose 0.5 g per kg body weight standardized cream (12% fat) 8 ml per kg body weight.

The infants were not allowed to eat or drink 4 hours after the test meal. Water was then allowed and after another hour the infants received a regular meal.

Capillary blood samples were taken at fasting 30

Altogether 21 regression analyses were performed (the three dependent physique variables in each of the seven age groups) and significant differences appeared between schools between social classes and between family positions on three occasions, once each. Since on average a significant result using a 5% level will be obtained by chance one in twenty times these results should be regarded with some circumspection. Quite different however is the effect on physique of menarcheal status. Ignoring the age groups 10 to 11 in which there are no girls who have achieved menarche, and 16+ in whom there is only one who has not in the remaining five groups the effect of menarcheal status on stature and weight is significant in each while its effects on ponderal index are significant in the three middle age groups 12 to 13, and 14 to 15, but not in the 11 to 12 or 15 to 16 groups. For the discrepancy in these two it seems that the height change is so pronounced relative to that in weight that the index remains relatively constant for it is in these two groups that the greatest differences in stature with menarcheal status occur (Table 9). Overall however the effects of menarcheal status on physique are consistent and pronounced. Girls who have attained menarche are consistently heavier and taller in each age group than those who have not the difference in height being particularly pronounced in the case of girls most extreme in maturation age. The magnitude of the overall effect on weight is perhaps not unexpected in view of the growth in the many different tissues that is occurring at puberty.

The absence of effect of any of the other variables is surprising. From the other surveys of physique in relation to environmental variables one might have expected some effect of social class (e.g. 1-7), or of family size (5) and these do not occur. Their absence may be partly due to the fact that even in a total sample of this size by the time the data are broken down by age groups and menarcheal status the subsamples are small and numbers insufficient to show any significant effect.

SUMMARY AND CONCLUSIONS

A survey of age at menarche how it is affected by family environment and how it affects physique, was carried out in 1967 on a large sample of schoolgirls in the urban industrial town of South Shields, County Durham. Age at menarche shows no independent effect of social class or of position in sibship but is strongly influenced by the size of family in which a girl grows up. Menarche is associated with consistent and pronounced increments in height and weight in the presence of which consistent effects of variables of the family environment on physique can be detected.

ACKNOWLEDGMENTS

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Table 2 Scheme of sampling and analytical methods after test meal

Analysis	Method (reference)	Total amount of blood required (ml)	Time of analysis after administration of the test meal (minutes)									
			30	60	90	120	180	240	300	360	480	600
Glucose	(5)	0.1	x	x	x	x	x					
D-xylose	(9)	0.1	x	x	x	x	x	x				
Triglycerides	(10)	0.4				x	x	x	x			
Vitamin A	(7)	0.4						x		x		x

x = analysis

grouped according to the ages of the infants and are presented in Fig 1. Because of the small number of cases and the great individual variability in the youngest normal infants a statistical analysis of the results was not possible. The individual variations were most pronounced in the youngest infants.

Glucose. The blood concentration rose to much higher values in the youngest infants than

in those being 13 to 20 months of age which is most likely explained by the fact that they received a greater amount of glucose in relation to their body weights. In 15 of the 16 infants studied the peak concentration was observed within 60 min after the administration of the test meal. In 12 of the 16 infants the blood concentration dropped to a level below 100 mg per 100 ml within 120 min.

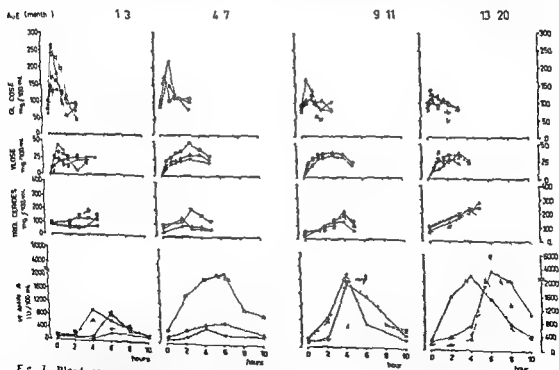


Fig 1 Blood concentrations of glucose, D-xylose, triglycerides and vitamin A after test meal given to normal infants of varying age. From the left to the right: \bigcirc - \bigcirc 1 month of age, \triangle - \triangle 1 1/2 months of age, \square - \square 2 months of age, \blacksquare - \blacksquare 3 months of age, \bullet - \bullet 3 months of age, \bigcirc - \bigcirc 4 months

of age, \triangle - \triangle 4 months of age, \square - \square 7 months of age, \bigcirc - \bigcirc 9 months of age, \triangle - \triangle 9 months of age, \blacksquare - \blacksquare 9 1/2 months of age, \bullet - \bullet 10 months of age, \bigcirc - \bigcirc 13 months of age, \triangle - \triangle 13 months of age, \blacksquare - \blacksquare 16 months of age, \bullet - \bullet 20 months of age.

Table 1 Clinical data in infants with malabsorption

Diagnosis	Case no	Sex	Age (mo)	Weight (kg)	Length (cm)	Remarks
Cystic fibrosis	1	♀	1½	2.4	48	Meconium ileus Chymotrypsin = 110-300 µg/g faeces Chlorides/sweat ^c = 128 mM
	2	♂	6½	6.5	64	Chymotrypsin = 26 µg/g faeces Chlorides/sweat ^c = 101 mM
	3	♀	19	9.6	80	Chymotrypsin = 20 µg/g faeces Chlorides/sweat ^c = 112 mM
Extrahepatic biliary atresia	4	♀	3	5.0	62	Atresia of D hepatici and \square choledochus ^d
	5	♂	4½	4.8	62	Atresia of D hepatici and \square choledochus ^d
	6	♂	10	5.3	62	Atresia of D hepatici and \square choledochus ^d
Intrahepatic cholestasis (Neonatal hepatitis)	7	♂	5	4.8	60.5	Persisting jaundice hepatosplenomegaly Congenital heart disease Operative cholangiography showed normal bile ducts
	8	♀	3	3.6	54	Jaundice hepatomegaly Susp congenital heart disease After 4 months of age regression of jaundice
	9	♂	13½	8.2	71	Persisting jaundice hepatosplenomegaly Operative cholangiography showed normal bile ducts
	10 ^a	♀	5½	4.7	59	Jaundice Hepatosplenomegaly Congenital heart disease Operative cholangiography showed normal bile ducts
Gluten induced enteropathy Before gluten free diet	11	♀	6	6.1	67	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	12	♂	9	8.0	69.5	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	13	♂	10½	7.3	70.5	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	14 ^b	♂	7	6.0	66	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation

^a Re-examined at 20 months of age when clinically healthy^b Re-examined at 13 months of age when clinically healthy Between the examinations the infant was on a gluten free diet^c Pilocarpin iontophoresis^d Verified at laparotomy and autopsy

RESULTS

Normal infants

The results of each of the parameters determined in the 16 normal infants have been

60 90 120 180 240 300 360 480 and 600 mm after the test meal. The amount of blood required and the time for analysis of each parameter is given in Table 2

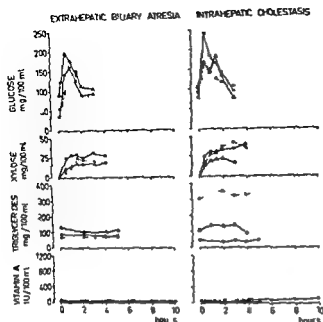


Fig 4 Blood concentrations of glucose D xylose triglycerides and vitamin A after test meal given to infants with malabsorption syndromes Extrahepatic biliary atresia ○—○ case 4 △—△ case 5 ●—● case 6 Intrahepatic cholestasis ○—○ case 7 △—△ case 8 ●—● case 9 For data on the patients see Table 1

infants In two patients the blood concentration of D xylose increased above 24 mg per 100 ml in the third the maximum concentration was 20 mg per 100 ml No increase in the blood concentration of triglycerides or vitamin A was seen in these 3 infants (Fig 4)

Intrahepatic cholestasis As can be seen from Fig 4 the maximum increase in blood glucose concentration was comparable to that found in normal infants The maximum blood concentration of D xylose was above 24 mg per 100 ml The blood concentration of triglycerides remained unchanged in two infants and increased in one infant 25% above the fasting level One of the infants had a high fasting level The blood concentration of vitamin A did not rise significantly in any of the three infants

Gluten induced enteropathy In only one of the three patients was the increase in blood glucose concentration 30 min after the test meal comparable to that seen in normal infants (Fig 3) In the other two infants the increase was only 6 and 14 mg per 100 ml above fasting level The maximum blood D xylose concentrations were 7 15 and 19 mg per 100 ml

ration of triglycerides did

not increase in two patients in the third the maximum increment was 43% above fasting level There was a slight increase of the blood concentration of vitamin A 6 hours after the test meal in only one patient

Consecutive studies in individual patients

The test meal was used to monitor intestinal absorption during the course of a disease and

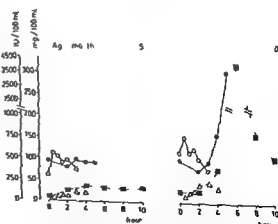


Fig 5 Blood concentrations of glucose D xylose triglycerides and vitamin A after test meal in an infant with intrahepatic cholestasis (case 10 Table 1) Symbols ○—○ glucose △—△ D xylose ●—● triglycerides ■—■ vitamin A

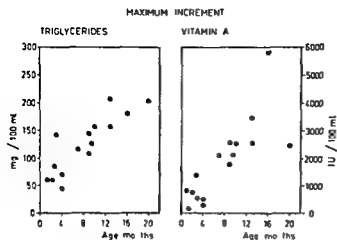


Fig 2 Maximum increases above fasting levels of triglycerides and vitamin A after test meal given to normal infants

D xylose The highest blood concentration was usually reached within 2 to 3 hours after the test meal. In all infants the maximum blood concentration was above 24 mg per 100 ml. In two of the youngest infants 1½ and 3 months of age there were two peaks.

Triglycerides The blood concentration reached a maximum within 4½ hours in 13 of the 16 infants. The maximum increment of triglycerides increased with increasing age (Fig 2). The percentage peak increment exceeded 63% in 15 of the infants studied.

Vitamin A Maximum blood concentration was reached within 4 to 6 hours after the test meal in all infants. The maximum increment of vitamin A increased with age (Fig 2). Below 4 months of age the maximum blood concentration never exceeded 1600 IU per 100 ml. On the other hand in all infants above this age the peak blood concentration exceeded this level.

Infants with malabsorption syndromes

Cystic fibrosis In the three infants studied the blood concentration of glucose and D xylose rose to the same level as found in normal infants of comparable ages. In two of the infants the blood concentration of triglycerides decreased initially. After 3 hours it returned to the fasting level in the first infant and to a value which was 13% above that level in the second infant. In the third patient there was an increase of 20 mg per 100 ml corresponding to an increment of 13%. No increase of the blood concentration of vitamin A was observed (Fig 3).

Extrahepatic biliary atresia The magnitude of the increases and the pattern of changes in blood concentrations of glucose in these 3 infants were comparable to those seen in normal

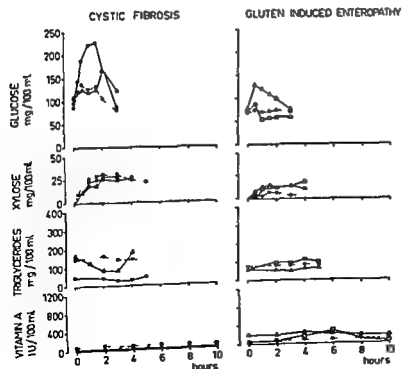


Fig 3 Blood concentrations of glucose, D xylose, triglycerides and vitamin A after test meal given to infants with malabsorption syndromes. Cystic fibrosis: ○—○ case 1, △—△ case 2, ●—● case 3. Gluten induced enteropathy: ●—● case 11, △—△ case 12, □—□ case 13. For data on the patients see Table 1.

bin-d tolerance test for D xylose and cream similar results have been obtained (4 b). The increases in the blood concentration of triglycerides were small in the youngest infants and in comparison to pathological states was not possible unless the percentage increment was calculated. In 15 of 16 infants this was found to be greater than 63% a value never reached in any of the infants with abnormal conditions studied. Disturbances in fat absorption in this age group however were readily detectable in the pattern of vitamin A responses (cf 4 a 13).

It is well known that infants with cystic fibrosis have an impaired absorption of triglycerides and vitamin A but normal absorption of D xylose. The expected results were thus obtained in our 3 patients studied with the test meal.

In the infants with extrahepatic biliary atresia and intrahepatic cholestasis a disturbance of fat absorption is expected due to an impairment of bile acid excretion to the intestine (12). This is well illustrated by the changes in blood concentration of triglycerides and vitamin A after the test meal (Figs 4 and 5). In some of the patients with cholestasis the blood concentration of D xylose was low compared to normal infants of the same age. Since D xylose tolerance test is thought to reveal mucosal damage which is not expected to occur in these conditions this finding is difficult to explain. However the fact that abnormal D xylose tolerance tests have been observed in adults with cirrhosis of the liver (15) may indicate that the absorption of D xylose is less efficient in liver disease. Bile acid excretion was not studied in the adults with cirrhosis of the liver. From our results obtained in the infant with intrahepatic cholestasis studied twice it is evident that the D-xylose triglycerides and vitamin A responses to the test meal became normal when bile acid excretion to the intestines had normalized spontaneously.

In gluten induced enteropathy with atrophy of the intestinal mucosa the absorption of fat as well as carbohydrates is known to be impaired. The present results using the combined

test meal are in agreement with those previously reported using single tolerance tests. The blood concentration of D-xylose never rose to values observed in normal infants. In the patient studied twice the absorption of fat and carbohydrates improved markedly after treatment with a gluten free diet.

There may be several advantages of the combined test meal compared to single tolerance tests. Since the test meal is comparable with a regular meal for the infant a prolonged partial fasting is avoided. It is considerably easier to follow the absorption of fat and carbohydrates during the course of a disease and during a therapeutic regimen when a series of single tolerance tests is replaced by one test e.g. a test meal.

SUMMARY

A test meal containing 2 g of glucose per kg body weight (minimum of 20 g), 0.5 g of D xylose per kg, 8 ml of standardized cream (12% fat) per kg and 7 500 IU of vitamin A palmitate per kg was administered through a nasogastric tube. During the next 10 hours capillary blood samples were obtained and analysed for glucose, D xylose, triglycerides and vitamin A using microanalytical methods.

The results in 16 normal infants aged 1 to 20 months are reported. The results for all parameters determined except D xylose were comparable to those previously reported using single tolerance tests. The variability related to age was pronounced for glucose, triglycerides and vitamin A.

In patients with malabsorption syndromes (cystic fibrosis, extrahepatic biliary atresia, intrahepatic cholestasis and gluten induced enteropathy) the results were also comparable with those earlier observed using single tolerance tests.

A combined test meal has certain advantages over the examinations now used in infants with symptoms suggesting a malabsorption syndrome. A series of tolerance tests is avoided and it is therefore easier to follow the absorption

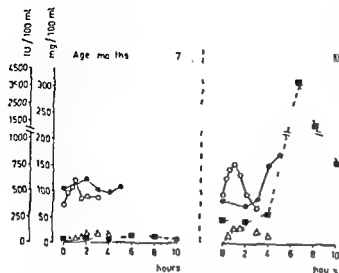


Fig 6 Blood concentrations of glucose D xylose triglycerides and vitamin A after test meal in an infant with gluten induced enteropathy (case 14 Table 1) Symbols ○—○ glucose △—△ D xylose ●—● triglycerides ■—■ vitamin A

to evaluate the effect of a therapeutic regimen. Two illustrative cases are shown in Figs 5 and 6.

In one infant with intrahepatic cholestasis (case 10 Table 1) the test meal was given at 5 months of age when almost all administered cholic acid ^{14}C was recovered in the urine, and again at 20 months of age when almost none was excreted in the urine (Fig 5). At 5 months of age the maximum increase in the blood D xylose concentration was below 24 mg per 100 ml, the increase in the blood concentration of triglycerides was only 19% and only a very slight (90 IU) increase in the blood concentration of vitamin A was observed. At 20 months of age the blood concentration of D xylose triglycerides and vitamin A rose to levels comparable with those seen in a normal infant of the same age.

One infant with gluten induced enteropathy (case 14, Table 1) (an intestinal biopsy specimen at 7 months of age showed typical mucosal atrophy) had only a slight increase in the blood concentration of D xylose triglycerides and vitamin A (Fig 6). The maximum blood concentration of D xylose was 11 mg per 100 ml 3 hours after the test meal. After 6 months on a gluten free diet, marked increase in the blood

concentration of D xylose, triglycerides, and vitamin A above fasting levels was found. The maximum blood concentration of D xylose (36 mg per 100 ml) was seen 2 hours after the test meal.

DISCUSSION

In normal infants the changes in the blood concentration of D xylose and especially of glucose, triglycerides and vitamin A following the test meal appeared to vary with age.

The high blood glucose concentrations in the youngest infants were probably a reflection of the relatively greater dose of glucose given per kg body weight. Influence of the other constituents in the test meal on the changes in the blood glucose concentration seems to be negligible, since their curves are otherwise comparable to those observed during simple glucose tolerance tests at these ages (2, 11).

The blood concentration of the D xylose seems, to some extent, to be influenced by the other components of the test meal. In the normal infants the blood D xylose concentration was low compared to those seen in ordinary D xylose tolerance tests (9), in addition the maximum blood concentration using the combined test meal occurred relatively late. It may be speculated upon if these findings can be explained at least partly by delayed gastric emptying which has been shown to occur in the presence of high concentrations of glucose (6). This may be one reason for the great individual variation in the youngest infants. A competitive inhibition of the intestinal active transport of D xylose by glucose may also be considered since such a phenomenon has been shown to occur *in vitro* (14).

The gradual increase of vitamin A response with the age of the infant as found in the combined test meal is in accordance with results obtained in single vitamin A tolerance tests (8, 16).

The variations in blood concentrations of triglycerides with the age of the infant were also considerable as shown in Fig 2. Using a com-

THE SIGNIFICANCE OF COPROANTIBODIES TO COW'S MILK PROTEINS

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Sensitivity to cow's milk protein appears to be an allergic phenomenon. It is diagnosed mainly on clinical grounds. The development of antibodies to cow's milk in the serum is a physiological process and does not in itself signify sensitivity to cow's milk (8, 20). If the titers of antibodies to cow's milk are very high, however, they usually do indicate some pathology. They may be found in milk sensitivity (9, 16, 24) in celiac disease (1, 15, 23) in mongolism (19) in dysautonomia (6) and in other chronic diseases (21). Since serological tests have not proved to be diagnostic (a search for milk antibodies in feces (coproantibodies) in children with gastrointestinal milk allergy seemed logical (2, 17)). We will report here on the value of coproantibodies in distinguishing gastrointestinal milk allergy from other conditions in which there are high levels of milk antibodies in serum. The immunoglobulin nature of the serum and coproantibodies to the proteins of cow's milk was also investigated.

MATERIAL AND METHODS

Four groups of children were investigated. Group 1 consisted of healthy infants under the age of 1 year seen during a prospective study of immunological responses to milk protein. Group 2 was comprised of children with a variety of acute or chronic conditions admitted to the Children's Department of

Shaare Zedek General Hospital. Group 3 consisted of children with mongolism or dysautonomia. Group 4 consisted of children with gastrointestinal milk sensitivity or celiac disease.

The children with milk sensitivity suffered from diarrhea and vomiting which ceased when cow's milk was replaced by a soybean milk preparation. The diagnosis was confirmed by three oral challenges with cow's milk and subsequent oral challenges with isolated milk proteins. Most of the patients have been described in detail elsewhere (9).

Five were sensitive to beta lactoglobulin, one to bovine serum albumin and one to casein. Celiac disease was diagnosed by fat balance, intestinal biopsy and response to gluten free diet.

Stools were centrifuged twice at 37 000 g for 45 min. Capillary blood was obtained in group 1 and venous blood in the other groups.

The following antigens were used—pasteurized whole milk centrifuged twice at 1 000 g for 10 min and once at 37 000 g for 30 min in the cold to remove fat and precipitable particles. Beta lactoglobulin crystallized and alpha lactalbumin purified were purchased from Pentex Co., Kan. Lake III bovine serum albumin crystallized and bovine gamma globulin from Armour Pharmaceutical Co., Chicago III. Casein 10 times crystallized was kindly supplied by Dr S. Saperstein, Pharmaceutical Division, the Borden Co., New York, N.Y.

For passive hemagglutination the technique of Royden (3) was employed with some modifications (13). Radio-immuno-electrophoresis was done by the method of Yagi et al. (25). The antigens were iodinated with ^{125}I by the chloramine T method. To every slide 0.1 ml of iodinated antigen was added. The concentration of the iodinated milk, bovine gamma globulin and beta lactoglobulin was 10–20 $\mu\text{g/ml}$ and that of alpha lactalbumin and bovine serum albumin 5–10 $\mu\text{g/ml}$. Each gamma of the iodinated antigen contained 5–10 μCi of ^{125}I . Weak antigen binding was shown by the arc of alpha globulin in some tested sera. A similar reaction was noted by Yagi

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of fat and carbohydrates during the course of a disease and during a therapeutic regimen

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Table 3 Incidence of precipitating serum and coproantibodies to milk proteins (all groups)

	Serum						Feces					
	Whole milk	ALA	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls	4/104	6/104	3/104	0/104	4/104	0/104	0/21	2/21	0/21	0/21	0/21	0/21
Other diseases ^a	2/14	3/14	1/14	2/14	1/14	0/14	0/34	2/34	0/34	0/34	0/34	0/34
Mongolism and dysautonomia	5/6	5/6	2/6	0/6	2/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Milk allergy												
Prolonged exposure	3/7	5/7	3/7	2/7	4/7	0/7	3/7	2/7	2/7	1/7	1/7	0/7
Short exposure	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Celiac disease	3/4	2/4	2/4	1/4	1/4	2/4	1/4	1/4	1/4	0/4	0/4	0/4

^a See first and last foot notes in Table 1

Numbers indicate positive reactions / total number of children examined

tions did not vary significantly from that in healthy controls. Over the age of 1 year coproantibodies were found in only one out of ten control cases tested.

The children with mongolism and with dysautonomia were analysed together as their immunological reactions were similar and we considered that aspiration of milk is a common factor in both. The incidence of coproantibodies was small while the incidence and the levels of antibodies in serum were abnormally high.

Table 2 describes hemagglutination reactions in milk allergy and celiac disease. When exposure to milk has been sustained for over 3 weeks high levels of antibodies are found in serum and feces in all cases. When exposure to milk did not exceed a few feeds the antibody response did not differ markedly from that of normal controls. In celiac disease mean antibody titers in serum and feces tended to be rather higher than in controls. We could find no evidence in any group that serum and coproantibodies were consistently directed against the same antigens. Furthermore antibody response gave no indication which of the milk proteins was responsible for symptoms of milk allergy.

The general pattern of the hemagglutination reactions was also reflected by our studies of serum and coproantibodies by precipitation in gel (Table 3). The very high incidence of serum

antibodies to milk in mongolism and dysautonomia is contrasted by the absence of coproantibodies in these conditions. On the other hand in gastrointestinal milk allergy prolonged exposure to milk resulted in a raised incidence of precipitating antibodies in feces and serum. This correlation between serum and coproantibodies held true for celiac disease as well.

The immunoglobulin nature of the antibodies to milk proteins were studied by radioimmunoelectrophoresis (Fig. 1). The responses in normal children and children with various acute and chronic conditions were similar and they are therefore considered as one group (Table 4). Serum antibodies were frequent and were of the IgG class. Coproantibodies to milk were found in 1 case only and were of the IgA class (Fig. 2).

A similar pattern prevailed also in mongolism and dysautonomia and was quite different from that obtained in milk allergy and celiac disease. Although in the last two conditions serum antibodies were also mainly of the IgG class it was only here that serum milk antibodies belonging to the IgA class could be found. Coproantibodies to milk were the rule among milk sensitive children after prolonged exposure. There were derived mainly from IgA and to a lesser extent from IgG.

The similarity of immunoglobulins in serum and feces was proven by immunodiffusion and

Table 1 Hemagglutinating serum and coproantibodies to milk protein groups 1 2 and 3

Group	Serum						Feces					
	Whole milk	ALA ^a	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls (1-12 months old)	18 ^b (9/10)	34 (10/10)	19 (8/10)	29 (9/10)	11 (9/10)	23 (10/10)	09 (6/15)	07 (4/15)	01 (2/15)	05 (4/15)	13 (11/15)	1 (6/15)
Other diseases ^c	19 (15/20)	23 (15/20)	23 (14/18)	26 (17/20)	16 (13/19)	18 (12/20)	06 (3/22)	09 (8/22)	07 (6/22)	04 (4/22)	16 (9/22)	11 (11/22)
Mongolism and dysautonomia	57 (6/6)	53 (6/6)	48 (6/6)	5 (6/6)	32 (6/6)	5 (6/6)	05 (1/6)	05 (1/4)	0 (0)	03 (1/6)	07 (3/6)	13 (4/6)

^a Abbreviations ALA = alpha lactalbumin BLG = beta lactoglobulin
BSA = bovine serum albumin BGG = bovine gamma globulin

^b Mean tube number and incidence of positive reactions are given Tube number refers to last test tube showing positive results Successive test tubes contained two-fold serial dilutions tube 1 being 1:20

^c Other diseases refers to children admitted to hospital with various acute and chronic conditions not associated with milk sensitivity

with other antigens (personal communication) and this is assumed to be non specific Our technique for radio immunoelectrophoresis was based on Yagis method (25) Details of this and the double micro immunodiffusion test in these experiments have already been published (9) Goat antisera against human IgA and IgM were purchased from Hyland Laboratories Los Angeles Calif Goat anti human IgG and goat anti human globulins were prepared in our laboratory The antisera to IgG IgA and IgM were tested against human serum by immunoelectrophoresis and found to be monospecific

Due to contaminants in a few of the milk proteins especially alpha lactalbumin and beta lactoglobulin as shown by the hemagglutination inhibition test this reaction was found to be not completely specific The data obtained by the radio immunoelectrophoresis and precipitation tests on the other hand proved to be more specific as the impurities did not affect the reactions

RESULTS

Passive hemagglutinating coproantibody titers in healthy controls are shown in Table 1 Coproantibodies were found even at the age of 1 month infants under 1 month were not examined The incidence of coproantibodies was slightly less than serum antibodies in the same group of patients The highest titer was 1:160 All cases with antibodies to cow's milk also had antibodies to one or more of the isolated proteins On the other hand not all individuals with antibodies to the isolated proteins had antibodies to whole milk The incidence of positive reactions in children under 1 year suffering from a variety of acute and chronic condi-

Table 2 Hemagglutinating serum and coproantibodies to milk proteins (group 4)

Group	No in group	Serum						Feces					
		Whole milk	ALA ^a	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Milk allergy													
Prolonged exposure	7	41 ^b (7/7)	54 (7/7)	5 (7/7)	53 (7/7)	47 (7/7)	4 (7/7)	33 (6/7)	4 (6/7)	31 (5/7)	2 (3/7)	4 (6/7)	33 (5/7)
Short exposure	5	2 (4/5)	22 (4/5)	3 (4/5)	28 (4/5)	2 (4/5)	24 (4/5)	02 (1/5)	0 (0)	04 (4/5)	02 (1/5)	0 (0)	2 (1/5)
Celiac disease	4	37 (4/4)	42 (4/4)	45 (4/4)	4 (4/4)	32 (4/4)	37 (4/4)	17 (2/4)	3 (3/4)	2 (2/4)	2 (2/4)	17 (3/4)	3 (2/4)

^a See first two foot notes to Table 1

Table 3 Incidence of precipitating serum and coproantibodies to milk proteins (all groups)

	Serum						Feces					
	Whole milk	ALA	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls	4/104	6/104	3/104	0/104	4/104	0/104	0/21	2/21	0/21	0/21	0/21	0/21
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Milk allergy												
Prolonged exposure	5/7	5/7	3/7	2/7	4/7	0/7	3/7	2/7	2/7	1/7	1/7	0/7
Short exposure	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Celiac disease	3/4	2/4	2/4	1/4	1/4	2/4	1/4	1/4	1/4	0/4	0/4	0/4

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Table 2 describes hemagglutination reactions in milk allergy and celiac disease. When exposure to milk has been sustained for over 4 weeks high levels of antibodies are found in serum and feces in all cases. When exposure to milk did not exceed a few feeds the antibody response did not differ markedly from that of normal controls. In celiac disease mean antibody titers in serum and feces tended to be rather higher than in controls. We could find no evidence in any group that serum and coproantibodies were consistently directed against the same antigens. Furthermore antibody response gave no indication which of the milk proteins was responsible for symptoms of milk allergy.

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The similarity of immunoglobulins in serum and feces was proven by immunodiffusion and

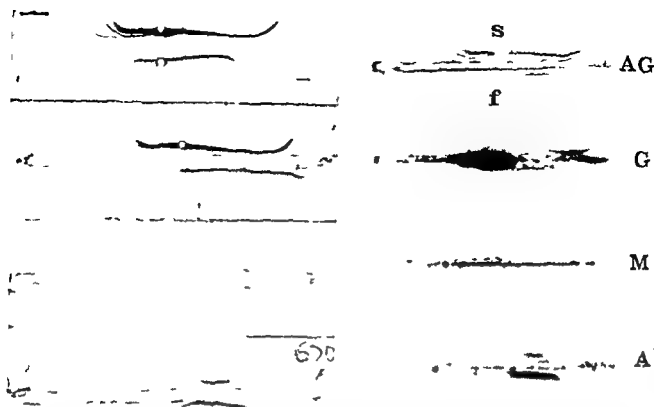


Fig. 1 On the left are shown radioimmuno-electrophoresis and on the right autoradiographs of serum (s upper well) and feces (f lower well) of an infant aged 3 months sensitive to cow's milk proteins. To upper trough (AG) anti human globulin was added to the other troughs monospecific antihuman IgG IgM and IgA (slides G M and A respectively) Slides

AG show that immunoglobulin and milk antibodies in serum belong mainly to IgG and in feces to IgA. This is confirmed by slides G and A. Very little immunoglobulin M was found in feces and no antibodies. Note the similarity of immunoglobulins in serum and feces.

lysis (Fig 3 top). All lines of the three fecal supernatants on the immunodiffusion slide showed lines of identity with IgG in the serum.

Two of these were identical with the IgA and one with IgM in the serum. In fecal supernatants an additional line appeared which bound

Table 4 Radioimmuno-electrophoresis showing immunoglobulin class of milk antibodies

Group	Serum			Feces		
	IgG	IgA	IgM	IgG	IgA	IgM
Healthy controls and children with other diseases ^{II}	16 ^a (9/12)	0 (0/12)	0 (0/12)	0 (0/12)	0.1 (1/12)	0 (0/12)
Mongolism and dysautonomia	3.2 (4/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
Celiac disease	2.6 (3/3)	0.6 (2/3)	0 (0/3)	0 (0/3)	1 (1/3)	0 (0/3)
Milk allergy						
Prolonged exposure	3.2 (5/5)	1.6 (3/5)	0 (0/5)	1 (3/5)	2.4 (4/5)	0.2 (1/5)
Short exposure	1.5 (2/2)	0.5 (1/2)	0 (0/2)	0 (0/3)	0 (0/3)	0 (0/3)

^a Numbers refer to intensity of reaction with an arbitrary scale of 0-5. Mean and incidence are given.

^{II} See last foot note to Table 1.

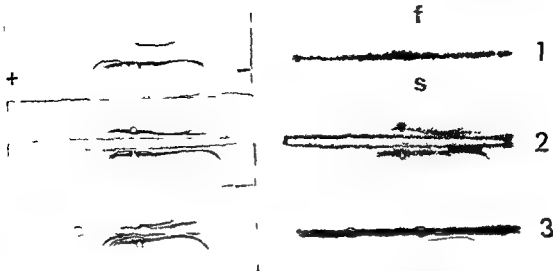


Fig 2 On the left are shown immunoelectrophoretic patterns of 1 normal child 2 child with milk sensitivity and 3 child with mongolism Upper well (f) fecal extract and lower well (s) serum from same individual The trough was filled with goat anti human serum globulin and radio-iodinated milk On the

right radio-immunoelectrophoretic pattern of same individuals Note presence of IgA and IgG antibodies to milk in serum and feces of case 2 IgG predominating in serum and IgA in feces In case 3 only serum IgG antibodies are present.

antigen thus making the radio-immunodiffusion method (as distinct from the radio-immunoelectrophoresis) unsuitable for detecting coproantibodies These lines were also observed by Krafí et al (18) Like others we found on immunoelectrophoresis of feces a substance running towards the anode (22) and this precipitate binds radio-activity It is possible that it is this substance which we found contaminating the precipitates in the radio-immunodiffusion reactions

DISCUSSION

The presence of hemagglutinating antibodies in feces to milk proteins is a physiological phenomenon (2) We found them to appear within a month after feeding of cow's milk They were present in three quarters of normal infants and their incidence dropped after the age of 1 year Similar findings have been observed in the serum (8)

The exact period required for the appearance of coproantibodies in our cases and whether they precede serum antibodies is not

known Observations with oral cholera vaccine suggest that coproantibodies appear earlier and disappear more rapidly (4 10) It appears that any food protein in the gastrointestinal tract may evoke an immunological response just as do pathogenic organisms The presence of precipitating or high levels of hemagglutinating antibodies is usually of pathological significance in the serum it may denote milk sensitivity celiac disease or aspiration (6) In feces it has previously been found only in milk and gluten sensitive infants (14 17) Both hemagglutination and precipitation in gel techniques produced a similar pattern of response in our groups of patients

Although we found abnormally high levels of antibodies in various pathological conditions we do not claim that these are sensitizing antibodies and as such responsible for the clinical picture They may be blocking antibodies and thus reduce the severity of the reaction

The presence of coproantibodies does not seem to be related to the presence of milk intake at the time of the test Two of our infants who had been off cow's milk for pro-

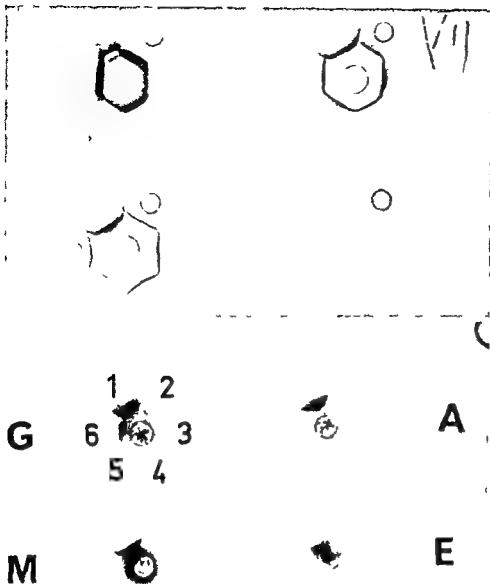


Fig 3 RID reactions of fecal supernatants (1 3 5) and sera (2 4 6) from three normal infants with goat antisera against IgG (G) IgM (M) IgA (A) and IgE (E diluted 1:30). Top: the immunodiffusion slide; bottom: the corresponding autoradiograph. Io

minated cows' milk was added to the wells of the antisera. Note the intensive false positive antigen binding on the precipitation lines of the fecal supernatants not related to immunoglobulins.

longed periods still had coproantibodies. On the other hand, two infants who were just beginning to tolerate cow's milk again had none. Most of the infants sensitive to milk in this study reacted clinically to beta-lactoglobulin but not to the other antigens given by mouth (9). This sensitivity is not reflected in the serum and coproantibody titers, which was high to a number of antigens. High titers of antibodies to antigens to which the patient is not clinically sensitive may be due to damage of the intestinal wall, allowing other antigens to

enter the blood stream and producing a response. Alternately, they may denote a state of increased immunological responsiveness in the gastrointestinal tract.

Abnormal levels of coproantibodies in our series were always associated with raised milk antibody titers in serum. In mongolism and dysautonomia, serum levels were high despite normal levels of coproantibodies. It has been our contention (6) that in these two conditions aspiration of milk is the cause of high titers of milk antibodies.

Antibodies may therefore be found in the serum and would be expected in the lungs but not in the gastrointestinal tract.

Serum antibodies of IgA type were found by radio-immuno-electrophoresis only in milk sensitivity and celiac disease. Although by radio-immunodiffusion we have found such antibodies even in healthy individuals their detection by the present method seems to denote pathology. The fact that we found coproantibodies to belong mainly to immunoglobulin A is in accordance with present concepts. IgA is the major immunoglobulin of the gastrointestinal mucosa (5, 7, 11) and is the main constituent of antibodies after local sensitisation (12).

SUMMARY

An attempt has been made to delineate further the significance of milk coproantibodies. The presence of precipitating and of high levels of haemagglutinating antibodies in feces was found only in patients with milk sensitivity or celiac disease. These cases showed also high levels of serum antibodies to milk. In mongolism and dysautonomia no abnormal titers of coproantibodies were found in spite of strong reactions in the sera.

Antibodies to milk were found by radio-immuno-electrophoresis to consist mainly of immunoglobulin A in feces and immunoglobulin G in serum. Serum milk antibodies of the IgA type were found only in patients with milk sensitivity or celiac disease.

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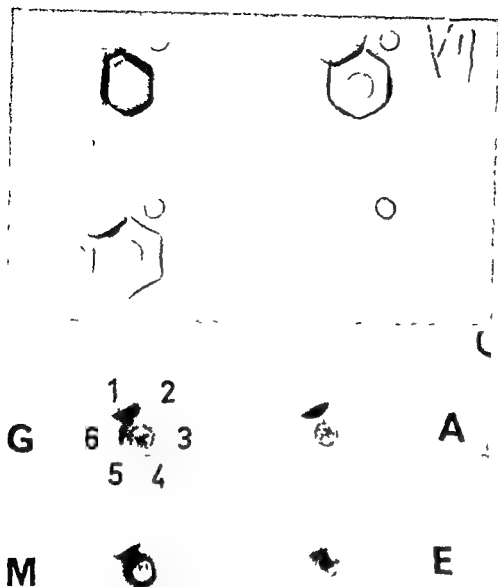


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CONTROL OF RESPIRATION IN NEWBORN BABIES

II The Development of the Thoracic Reflex Response to an Added Respiratory Load

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In a recent publication (2) it was shown that the strength of the Hering Breuer inflation reflex in babies (i.e. inhibition of inspiration in response to lung distension) varies with age being very weak at a postmenstrual age of 32 weeks to increase to a maximum strength at 36 to 38 weeks and then to decrease in strength again. Whereas the primary weakness of the reflex at an early postmenstrual age may simply imply that some of its linkages fail at that stage the decreasing strength seen after 38 weeks is more difficult to explain. Hypothetically the reason for this decline might be that other reflexes such as e.g. the thoracic spinal reflexes related to breathing then are becoming stronger and thus relatively more important.

The existence of thoracic spinal reflexes in the nervous control of breathing has recently been demonstrated. Campbell (4) found that an increase of the respiratory load leads to an increase of the mechanical power generation of the respiratory muscles and Sears (see 10) and Euler and co-workers (see 6) later showed that such a response is reflexly mediated via thoracic dorsal root afferents from intercostal muscle spindles. The aim of the present investigation was to study this reflex system in babies of different postmenstrual ages by recording the effect on the intrapleural pressure swings of an added respiratory load.

MATERIAL AND METHODS

Eight "normal" infants of varying gestational age were studied and the experiments were performed at varying times postnatally corresponding to 30½ to 42 weeks of postmenstrual age (Table 1). Four of the babies were studied more than once (Table 1). The calculation of the postmenstrual age (the sum of gestational and postnatal ages) was based on the mothers last normal menstruation and the length and weight of the baby at birth. There was a good correlation between the postmenstrual age and the neurological behaviour and reflex pattern (8) in all the babies studied.

An estimation of the capacity of the thoracic reflex system was achieved by recording the amplitude change of the intraesophageal pressure swings caused by occlusion of the airways. Since the intraesophageal pressure equals the intrapleural pressure (7) a gradual change in amplitude of the former caused by airway occlusion can be assumed to reflect a change in the power generation of the respiratory muscles. The intraesophageal pressure was recorded via an open ended saline filled polyethylene catheter introduced via a nostril to the lower third of the esophagus and connected to a pressure transducer (T. Ljungström Sweden). The pressure swings were displayed on a Mingograph Recorder (Elema Schonander Sweden). A rubber face mask (no. 0 Rendall Baker) slightly modified for the pressure recording was placed appropriately and sealed around the mouth and nose of the baby. By simply occluding the opening of the mask a sudden increase of the respiratory load was achieved. The occlusions were undertaken from the height of expiration or inspiration and only when the baby was breathing calmly. When the occlusion was made at the height of an inspiration the Hering Breuer inflation reflex was of course also evoked (cf 2).

The rectal temperature was controlled throughout the experiments and found to remain constant at

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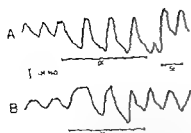


Fig 3 Tracings of the intrasophageal pressure swings during spontaneous breathing and airway occlusion (i.e. occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 37 weeks. In A airway occlusion begins at the height of an expiration. In B at the height of an inspiration. Inspiration deflection downwards.

an inspiration. In both cases the intrasophageal pressure amplitude decreases as a consequence of the occlusion. Moreover the respiratory rate increases slightly although in B where the occlusion is performed at the height of an inspiration a slight prolongation of the first consecutive expiratory phase is seen (Hering Breuer inflation reflex of 2). Fig 2 shows the corresponding tracings from three other babies 31 (A) and 32 (B, C) weeks of age and illustrates the variability of the changes in respiratory rate in response to airway occlusion at this developmental stage. In all cases the occlusion is performed at the height of an expiration and as can be seen the amplitude of

the pressure swings decreases as a consequence of the occlusion. However in the first case (A) a few shallow breathes is followed by complete respiratory arrest lasting until the face mask is removed. In the second case (B) a rapid fibrillating breathing pattern occurs and in the third case (C) breathing becomes highly irregular. Fig 3 finally shows the respiratory responses to airway occlusion in a 37 weeks old baby. Both when the occlusion is performed at the height of an expiration (A) and at the height of an inspiration (B) a pronounced gradual increase of the amplitude of the pressure swings takes place. This response is similar to that of adult animals and man which reflects an increasing power generation of the respiratory muscles in response to the added load (4, 6, 9). There is also a decrease in respiratory rate which is more pronounced in B where the Hering Breuer inflation reflex is also evoked (cf 2).

The change with age of the respiratory muscle response to an added load has been illustrated graphically in Fig 4. Here the relative increase in amplitude caused by the occlusion has been plotted against the postmenstrual age of the baby at the time of the experiment. Except for a decrease between 34 and 37 weeks the response is seen to increase gradually in strength between 30 and 42 weeks of age. As can be

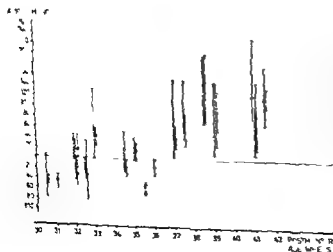


Fig 4 Diagram showing the strength of the response to airway occlusion (ordinate) in 8 babies studied at 19 different occasions plotted against the postmenstrual age of the babies at the time of the experiment (abscissa). The strength of the response to airway occlusion has been represented as the mean relative change in amplitude of the intrasophageal pressure swings of the 3 (occasionally 2) first breathing cycles during occlusion as compared to the mean amplitude of 5 breathing cycles immediately preceding the occlusion. The filled circles derive from experiments where the occlusion has been undertaken from the height of an expiration and the open circles from experiments where the occlusion has been undertaken from the height of an inspiration. The vertical lines represent the ranges.

Table 1 *Data of infants examined*

Case	Birth weight (g)	Length at birth (cms)	Gestational age (weeks)	Postmenstrual age at experiment (weeks)
I female	1 420	40	31-32	32-33 34-35 35-36 37-38
II female	3 220	49	37	38-39
III male	1 630	39	30	30-31 31 32 35-36
IV male	4 420	52	40	41
V male	3 230	52	37	37
VI female	1 030	37	30	32 33 35 36 39 41
VII female	3 500	49	39	39
VIII male	3 160	51	42	42

36-37°C. The smaller babies were studied in incubators. Since the face mask adds to the dead space of the airways it was kept to the face for only a few minutes each time.

RESULTS

It was soon found out that the respiratory response to an added load varied with the postmenstrual age of the baby. Thus, in the youngest babies studied, the amplitude of the intraesophageal pressure swings remained constant or even decreased on airway occlusion, whereas in the older babies the pressure amplitude in-

creased instead, this increase being larger the older the baby. Moreover, the older the baby, the more constant was the change in respiratory rate caused by airway occlusion. These findings are illustrated in Figs 1-3.

Fig. 1 shows the tracings of the intraesophageal pressure swings during spontaneous breathing and the effect of airway occlusion in a 32-week-old baby. In A, occlusion of the opening of the face mask is performed at the height of an expiration; in B, at the height of

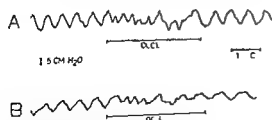


Fig. 1 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (i.e. occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 32 weeks. In A, airway occlusion begins at the height of an expiration; in B, at the height of an inspiration. Inspiration deflection downwards.

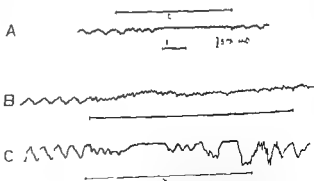


Fig. 2 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion from the height of an expiration in three babies: 31 (A) and 32 (B, C) weeks of postmenstrual age. Inspiration deflection downwards.

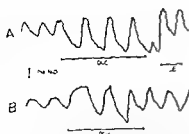


Fig 3 Tracings of the intrasophageal pressure swing during spontaneous breathing and airway occlusion (i.e. occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 37 weeks. In A airway occlusion begins at the height of an expiration. In B at the height of an inspiration. Inspiration deflection downwards

an inspiration. In both cases the intrasophageal pressure amplitude decreases as a consequence of the occlusion. Moreover the respiratory rate increases slightly although in B where the occlusion is performed at the height of an inspiration a slight prolongation of the first consecutive expiratory phase is seen (Hering Breuer inflation reflex cf 2). Fig 2 shows the corresponding tracings from three other babies 31 (A) and 32 (B, C) weeks of age and illustrates the variability of the changes in respiratory rate in response to airway occlusion at this developmental stage. In all cases the occlusion is performed at the height of an expiration and as can be seen the amplitude of

the pressure swings decreases as a consequence of the occlusion. However in the first case (A) a few shallow breathes are followed by complete respiratory arrest lasting until the face mask is removed. In the second case (B) a rapid fibrillating breathing pattern occurs and in the third case (C) breathing becomes highly irregular. Fig. 3 finally shows the respiratory responses to airway occlusion in a 37 weeks old baby. Both when the occlusion is performed at the height of an expiration (A) and at the height of an inspiration (B) a pronounced gradual increase of the amplitude of the pressure swings takes place. This response is similar to that of adult animals and man which reflects an increasing power generation of the respiratory muscles in response to the added load (4 & 9). There is also a decrease in respiratory rate which is more pronounced in B where the Hering Breuer inflation reflex is also evoked (cf 2).

The change with age of the respiratory muscle response to an added load has been illustrated graphically in Fig. 4. Here the relative increase in amplitude caused by the occlusion has been plotted against the postmenstrual age of the baby at the time of the experiment. Except for a decrease between 34 and 37 weeks the response is seen to increase gradually in strength between 30 and 42 weeks of age. As can be

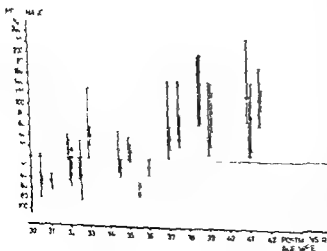


Fig 4 Diagram showing the strength of the response to airway occlusion (ordinate) in 19 babies studied at 19 different occasions plotted against the postmenstrual age of the babies at the time of the experiment (abscissa). The strength of the response to airway occlusion has been represented as the mean relative change in amplitude of the intrasophageal pressure swings of the 3 (occasionally 2) first breathing cycles during occlusion as compared to the mean amplitude of 5 breathing cycles immediately preceding the occlusion. The filled circles derive from experiments where the occlusion has been undertaken from the height of an expiration and the open circles from experiments where the occlusion has been undertaken from the height of an inspiration. The vertical lines represent the ranges.

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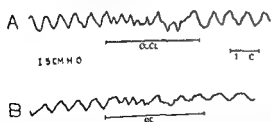


Fig 1 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (i.e. occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 32 weeks. In A airway occlusion begins at the height of an expiration. In B at the height of an inspiration. Inspiration deflection downwards.

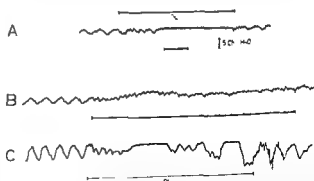


Fig 2 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion from the height of an expiration in three babies 31 (A) and 32 (B, C) weeks of postmenstrual age. Inspiration deflection downwards.

from the height of an expiration when the pulmonary tension receptors are thus differently stimulated which further indicates that the response in the old babies is not evoked via pulmonary vagal afferents. Also hypercapnia and hypoxia as the cause of the increase in amplitude of the first few breaths after occlusion seems unlikely as pointed out by earlier authors (4).

A similar development of the response to an added respiratory load as described in the present work for newborn babies has been shown to occur postnatally in kittens (9). In such animals the development of the respiratory muscle response to airway occlusion has been tentatively related to the development of the nerve fibres and especially to the innervation of the intercostal muscle spindles (9). The present finding in the youngest babies of a decrease of the amplitude of the intraesophageal pressure swings in response to airway occlusion (Figs 1 and 2) indicates that, the addition of a respiratory load inhibits the activity of the respiratory muscles at that stage. Such an inhibition has also been demonstrated in newly born kittens and again related to the innervation of the intercostal muscle spindles (9). Thus the findings can be explained if the intercostal muscle spindles—like hind leg muscle spindles of newborn kittens (see 11)—lack an efferent "gamma" innervation at that stage. Then the spindles function as passive stretch receptors only and will not respond to an added load. Instead the inhibitory influence from the Golgi tendon organs might dominate thus explaining the finding of an inhibition of the muscle activity in response to the airway occlusion (cf 9).

In an earlier publication (2) it was shown that the Hering Breuer inflation reflex is very weak in babies 32–34 weeks of postmenstrual age. This finding was principally confirmed in the present investigation (Figs 1B and 5). However the fact that occlusion in maximal expiration (which prevents lung expansion and activation of the Hering Breuer inflation reflex) at that stage produced an irregular breathing

pattern and sometimes respiratory arrest (Fig 2) still shows that this reflex although weak is of primary importance for a proper functioning of the respiratory center as is the case in newborn animals (9).

An interesting observation is that the development of the strength of the thoracic reflex to an added load shows a dip around 36–38 weeks or when the Hering Breuer reflex reaches its maximum strength (Figs 4 and 5). This might indicate that the two reflexes in some way compete. Nothing seems to be known about the influence from the intercostal and thoracic wall receptors on supraspinal centers or of a possible competition between thoracic and pulmonary reflexes but if such an interaction does exist, this could also explain why the Hering Breuer inflation reflex decreases in strength after 38 weeks of age when the thoracic reflex continues to increase in strength.

SUMMARY

The respiratory response to an added load recorded as the change in amplitude of the intraesophageal pressure swings caused by airway occlusion has been studied in 8 babies 30½ to 42 weeks of postmenstrual age. With increasing age the response to such an occlusion was found to increase gradually and it is concluded that this is a reflection of an increasing maturation of a thoracic respiratory reflex. The results are related to similar findings in developing animals and to earlier studies on the development of the Hering Breuer inflation reflex in babies.

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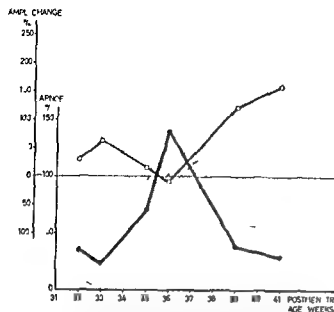


Fig 5 Diagram showing the development of the strength of the Hering Breuer inflation reflex (filled circles) and of the strength of the thoracic reflex response to an added load (open circles) in one and the same baby studied at six occasions between 32 and 41 weeks of postmenstrual age. The strength of the Hering Breuer inflation reflex was calculated as the relative increase in length of the first breathing cycle following airway occlusion from the height of a normal inspiration (see Ref 1). The strength of the thoracic reflex response was calculated as the mean relative increase in amplitude of the intraoesophageal pressure swings of the first 3 breaths following airway occlusion from the height of a normal expiration. Shaded areas represent ranges.

seen, in this respect the results are the same irrespective of whether occlusion was performed from the height of an expiration (filled circles) or an inspiration (open circles). Since the response in the older babies is principally the same as in adult animals and man where it has been attributed to a thoracic spinal reflex (4, 6, 9) it may be concluded that the strength of this reflex increases with postmenstrual age of the baby.

In one baby estimations both of the strength of the Hering Breuer inflation reflex and of the strength of the amplitude response to an added load i.e. of the thoracic reflex were made at different occasions between 32 and 41 weeks of postmenstrual age. The results plotted in Fig 5, show the same principal development of the reflexes in this single baby as the plottings of all individual values (see Fig 3 and Ref 2).

It may be pointed out that here also the development of the amplitude response shows a dip around 36 weeks or when the Hering Breuer inflation reflex reaches its maximum strength.

DISCUSSION

In adult animals and man airway occlusion is known to provoke reflexes both from the lungs via the vagus nerves and from the thoracic wall via the intercostal nerves (3, 4). Whereas the vagal reflexes seem to affect mainly the respiratory rate (3, 12) the thoracic reflexes have been shown to affect the power generation of the respiratory muscles (4, 6, 10). The present finding of an increase with age of the power generation of the respiratory muscles in response to airway occlusion (Figs 1, 3, 4 and 5) thus indicates that the importance of the thoracic reflex system increases with postmenstrual age. It may be argued that since the face mask (volume 10 ml) used was the same for all babies a relatively larger dead space and thus a smaller elastic load was added to the smallest babies which may then explain the feeble response in these individuals. Since however the elastic load produced by airway occlusion is not related to the dead space only but rather to the total lung volume which was probably less than 100 ml in the smallest babies and more than 300 ml in the biggest ones (cf 1 and 5), the added load must rather have decreased with age instead. Another argument might be that the amplitude response is a consequence of an altered vagal afference due to the airway occlusion. Thus when the airways are occluded from the height of expiration the lungs are prevented from expansion during the next inspiratory effort so the Hering Breuer inhibition reflex is not activated. Although this explains the prolonged durations of the inspiratory phases in the older babies (Fig 3) it does not explain the gradual increase in intraoesophageal pressure amplitude. Moreover the present results show that the amplitude response was essentially the same when the occlusion was performed from the height of an inspiration as

THE COMBINED XYLOSE DISACCHARIDE TOLFRANCE TEST ITS APPLICATION FOR DIAGNOSING DISACCHARIDASE DEFICIENCY

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The main cause of a flat blood glucose curve after ingestion of a disaccharide e.g. lactose or sucrose lies in defective digestive absorptive function (6) low gastric or intestinal motility probably being of less importance (1). If we wish to differentiate a disaccharidase deficiency from other causes of defective sugar absorption at least two tests e.g. a disaccharide test and a test with its component monosaccharides are needed (4 11 21).

We felt that the disaccharide test might be improved by administering the disaccharide in combination with the pentose *d*(+) xylose. Xylose absorption in adults and children is well documented and the xylose test is often used in diagnosing malabsorption (2 3 5 12 14 15 16). By measuring simultaneously xylose and disaccharide absorption it should be possible to assess the digestion of the tested disaccharide in a single test because xylose being absorbed by the same mechanism as other sugars with the pyranose ring structure such as glucose and galactose (19) would serve as an indicator for the absorptive function.

In this article the results of combined xylose disaccharide tolerance tests in children with sucrase isomaltase deficiency are described. For comparison the results of the same tests in normal children are given. Our experience with the xylose test is discussed briefly.

MATERIAL AND METHODS

The study comprised two groups of children (I) 61 "normals" and (II) six children with a sucrase isomaltase deficiency. The normal children were a heterogeneous group including some children with a history of diarrhoea. Not included were children with proven villous atrophy of jejunal mucosa, disaccharidase deficiency, liver disorders or inborn errors of metabolism. The diagnosis sucrase isomaltase deficiency of the second group had been confirmed by enzymic assay in a biopsic sample of jejunal mucosa; the pertinent data are given in Table 1. The patients were biopsied with the twin hole capsule of Sebus (20). No medication was given during the experiments. *d*(+)-Xylose was administered alone or combined with glucose, fructose, lactose, maltose, sucrose or starch. The doses used were 0.5 g xylose and 2 g of the other sugars per kilogram body weight with a maximum of 25 and 40 g respectively. The required amount of xylose was dissolved in a 10% solution of the test sugar. Thus the xylose concentration was 2.5% at a total dose of 12.5 g or lower and 5% at the maximum total dose of 25 g xylose and 50 g other sugar in 500 ml water. After ingestion of the sugar solution capillary blood samples were taken at 30 min intervals during 3 hours. No attention was given to the position or the activity of the child. The fasting period prior to the test was usually 14 hours; infants were fasted for at least 8 hours. Blood xylose was estimated according to Roe & Rice (18). Blood glucose was estimated with glucose oxidase (reagent set TC M of Boehringer Mannheim Germany).

RESULTS

Fig. 1 shows the mean blood xylose levels (± 2 SD) in 90 oral tolerance tests performed

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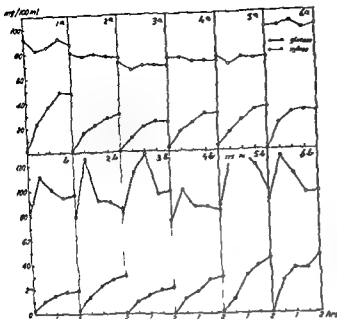


Fig 3 Xylose sucrose tolerance test (1a-6a) and xylose lactose tolerance test (1b-6b) in six children with sucrase isomaltase deficiency Nos 1 and 2 are brothers. The last hour of the test has been omitted since it yields no relevant information.

there are considerable variations in xylose levels during the tests (Fig 1) the xylose curves of each child (Fig 2) are much less divergent. This confirms the good reproducibility of the test as reported by Brien *et al* (3). Incidentally the data indicate that the addition of another sugar to the xylose test has only a relatively small competitive effect on xylose levels. Likewise when xylose was combined with starch boiled in water (10% solution) we found the ensuing xylose curve to be only slightly affected (experiments on three children). Benson (2) found a distinct competition effect when he added xylose to a meal. For the present purpose a competition effect if present is of little consequence since xylose absorption would be enhanced rather than depressed by a decreased glucose release from the ingested disaccharide.

Beck *et al* (1) did not find any influence of a prolonged gastric emptying time on xylose levels except with complete or almost complete pyloric obstruction. This agrees with our findings: we observed abnormally low xylose levels only in two infants with pyloric stenosis; the xylose curves of three other children with proven delayed emptying of the stomach but

normal absorption being essentially normal. A flat xylose curve after xylose loading is almost always caused by a substantially reduced absorptive function (5, 12, 14, 15). We found significantly low xylose levels in three out of six celiac children.

The xylose curves of the sucrase isomaltase deficient children were all in the normal range. The flat glucose curves after sucrose loading are in marked contrast with the normal xylose rise (Fig 3). From the distinct xylose rise one must conclude that the glucose absorption mechanism is intact. So the flat glucose curve must be due to impaired sucrose hydrolysis i.e. to low sucrase activity. Lactase activity was normal as follows from the result of the combined xylose lactose test in the same children.

Thus the combined xylose disaccharide test provides a dependable means to diagnose in a single test a deficiency of the disaccharidase concerned. It also allows a more certain interpretation of the disaccharide test as the latter is sometimes inconclusive and may even be at variance with the disaccharidase activity of biopsy specimens (10, 17).

We derive the following interpretation of the test from our experiments: (1) a normal rise of

Table 1 Data of six children with disaccharidase deficiencies

Patient	Sex	Age	Enzyme activities ^a in jejunal mucosa				
			Sucrase	Isomaltase	Palatinase	Maltase	Lactase
R T	♂	7	19	—	0.2	60	43
A T	♂	5	0.0	—	0.0	73	43
C R	♂	2	0.0	0.5	—	3.6	35
M E	♂	1	0.0	0.9	—	4.1	70
W L	♀	8	0.3	—	0.2	15	9.6
J K	♂	14	0.1	0.9	—	4.8	6.2
Normal			4.0-17.4 ^b	3.8-16.8 ^b	1.5-4.0 ^c	15.8-55.6 ^b	1.3-11.8 ^b

^a μ Moles substrate hydrolyzed per minute and per gram tissue (palatinase method of Dahlquist (7) other disaccharidases method of Eggermont (8))

^b Range of a control group of 35 children at ages from 1 month up to 14 years Data from Eggermont & Hers (9)

^c Data from Dahlquist (7)

on the control groups of 61 children Fig 2 shows the results of both a xylose test and a combined xylose glucose or xylose-sucrose test performed on four children of the control group For each child the xylose curves, whether combined with another sugar or not, were more similar than the curves of different children For greater clarity the glucose data have been omitted from the Fig 2, but in all combined tests glucose rose normally The results of the combined xylose disaccharide tests performed on six children with sucrase isomaltase deficiency are presented in Fig 3 When xylose was combined with sucrose the xylose level rose and the glucose level remained constant (Fig 3 upper part) but simultaneous oral administration of xylose and lactose resulted in a concomitant rise of both xylose and glucose (Fig 3 lower part)

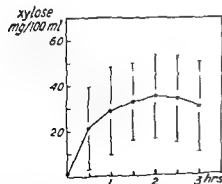


Fig 1 Mean blood xylose levels (± 2 SD) of 90 oral tolerance tests performed on 61 normal children Xylose was administered alone or combined with glucose fructose sucrose or starch

DISCUSSION

Our xylose dose was the same as used by Jones & Di Sant Agnese (13) and approximates the dose used by Ingemar et al (12), Meeuwisse & Dano (15), and Møllerberg & Soderhjelm (16) viz 15 g per square meter of body surface We did not observe any side effects of the test such as one or more loose stools except during the xylose sucrose test in the sucrase isomaltase deficient children Meeuwisse & Dano (15) who used a 10% xylose solution saw these also in other children

While among the control group consisting of children without apparent absorption defect

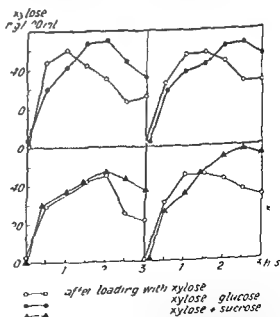


Fig 2 Xylose tolerance test in four normal children

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blood xylose in combination with a flat glucose curve (Fig 3) indicates a disaccharidase deficiency since the xylose rise renders a defective sugar absorption mechanism unlikely, (II) if both glucose and xylose rise normally, impaired hydrolysis of the tested sugar is excluded, (III) if the xylose rise is small, an impaired absorptive function is likely, though nothing can be said about its cause. In our experience, the result of the combined xylose-disaccharide test is seldom ambiguous.

In conclusion, we recommend the addition of xylose to the disaccharide tolerance test if a child is suspected of having a disaccharidase deficiency.

SUMMARY

Six children with a sucrase-isomaltase deficiency were subjected to an oral tolerance test with sucrose or lactose combined with $d(+)$ xylose.

Simultaneous determination of a xylose curve allowed a more certain interpretation of the blood glucose curve. A flat glucose curve combined with a normal xylose rise points to an impaired enzymic hydrolysis rather than to a defective absorption of its component monosaccharides. Compared with the normal range ± 2 S.D. derived from 90 xylose curves of 61 normal children the sucrase-isomaltase deficient children showed normal xylose curves.

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STABILITY OF ACID PHOSPHATASE OF FETAL RED BLOOD CELLS DURING INCUBATION WITH ACETYLPHENYLHYDRAZINE

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Maintenance of the sulfhydryl (SH) groups of erythrocyte components in the reduced state has been considered important for the functional and structural integrity for the normal survival of the red blood cell (8 9 10). It has been shown previously that during incubation with acetylphenylhydrazine (APH) or with oxidized glutathione (GSSG) the red blood cell acid phosphatase of adult subjects undergoes striking changes in its electrophoretic properties associated with a marked reduction in enzymatic activity (2-4). Since this enzyme is SH dependent, the investigation of its properties in the red blood cells (RBC) from newborn infants could contribute to a better understanding of some aspects of RBC metabolism during this early period of life. In particular it could help to define the relationship between the increased instability of reduced glutathione (GSH) and the high vulnerability of neonatal RBC to oxidative damage.

In a previous study (5) we have shown that the acid phosphatase of fetal RBC is less stable than that of maternal RBC during incubation with APH. The results of the present study confirm these observations and show a significant correlation between the fall in GSH concentration and the diminution in acid phosphatase

activity in the red cells. This study also shows that on the 3rd day of life the RBC acid phosphatase is more stable than at birth.

MATERIAL AND METHODS

The blood samples for the study were obtained from 20 normal full-term newborn infants and their mothers and from 13 normal premature infants (gestational age <35 weeks weight <23 kg) and their mothers. Blood was withdrawn with plastic syringes from the umbilical vein at birth and in 10 infants also from the jugular or femoral vein at 3 days of age. Blood from mothers was obtained from the antecubital vein shortly before or after delivery and in 10 of the same mothers also 3 days post-partum. All infants were Rh and ABO compatible with their mothers; the direct Coombs test on cord blood was negative and serum bilirubin levels at 3 days of age were within normal limits. The RBC of 8 normal adults (4 males and 4 females) were also studied.

The blood was immediately transferred into heparinized test tubes, mixed and centrifuged at 3000 rpm for 10 min. After centrifugation the plasma and buffy coat were removed and the RBC were washed 3 times with cold saline and then resuspended in saline to a final hematocrit of 50%. The suspensions of neonatal and maternal RBC with and without APH (4 mg/ml of suspension) were then incubated aerobically and with continuous agitation for 2 hours at 37°C. In 6 experiments the simultaneous changes in GSH concentration and acid phosphatase activity were followed during incubation by withdrawing small aliquots of RBC suspension at fixed time intervals (0 30 45 60 and 120 min).

Table 1 Acid phosphatase activity of red blood cells from different subjects after incubation with acetylphenylhydrazine for 2 hours at 37 C

The enzymatic activity has been expressed as percent of the activity before incubation

Group studied	No of subjects	Residual enzymatic activity (average \pm SD)	% Difference	Significance of the difference ^a
Full term infants at birth	20	43.2 \pm 8.6	27.8	$p < 0.001$
Mothers at delivery	20	55.2 \pm 10.5		
Full term infants at 3 days	10	43.3 \pm 10.0	20.9	$p < 0.001$
Mothers at 3 days post partum	10	52.3 \pm 9.6		
Full term infants at birth	10	39.8 \pm 8.1	8.6	$p < 0.05$
Full term infants at 3 days	10	43.3 \pm 10.0		
Mothers at delivery	10	56.3 \pm 10.6	7.0	Not significant
Mothers at 3 days post partum	10	52.3 \pm 9.6		
Premature infants at birth	13	47.6 \pm 11.5 ^b	12.4	$p < 0.01$
Mothers at delivery	13	53.5 \pm 13.3		

^a The Student's *t* test was calculated for paired observations

^b This value does not differ significantly from that of full term infants at birth

sured according to Beutler et al (1) and the acid phosphatase activity was measured using a modification of the method described by Tormani (10, 11). 4 determinations of RBC acid phosphatase before and after 2 hour incubation with APH were performed in normal adults. Variance analysis of the results showed that the variability of the method was significantly lower than the variability among individual subjects ($p < 0.001$). The data also showed that there were no appreciable differences in 2 consecutive measurements performed in the same subject at intervals of 8 days.

RESULTS

The results obtained in 20 full term and in 13 premature infants and in their mothers are shown in Table 1.

The inactivation of RBC acid phosphatase during incubation with APH was significantly greater in the erythrocytes of full term and premature infants than in maternal red cells ($p < 0.001$ and < 0.01 respectively).

In those infants studied also at 3 days of age RBC acid phosphatase was significantly more stable than at birth ($p < 0.05$). However the enzyme was still significantly less stable than in maternal blood. It should also be noted that most of the infants studied at 3 days were among those who had showed a marked in

stability of acid phosphatase at birth when compared with their mothers.

The results obtained in mothers on the 3rd post partum day were not significantly different from those obtained in the same subjects at the time of delivery. The acid phosphatase stability of RBC from premature infants at birth was not significantly different from that of RBC of full term newborns. In Table 1 the residual acid phosphatase activity after incubation with APH has been expressed as per cent of the activity before incubation. When RBC were incubated for 2 hours without APH there was a slight fall in acid phosphatase activity which was similar in maternal and fetal RBC (Fig 1). When the residual acid phosphatase activity after incubation with APH was expressed as per cent of the activity in the same RBC incubated for 2 hours without APH the results did not change and the differences mentioned above were present to the same degree.

Fig 1 shows the results of 6 experiments where the fall in GSH and in acid phosphatase activity were determined simultaneously in fetal and maternal RBC during incubation with and without APH. The fall in acid phosphatase activity followed with a slight delay that of GSH

both in maternal and fetal RBC. The GSH level began to decrease earlier in fetal than in maternal RBC and the difference between the 2 bloods was already significant after 30 min of incubation. Also the fall in acid phosphatase activity of fetal RBC preceded that of maternal RBC, however, the difference became significant only after 45 min of incubation.

Fig 2 shows a clear correlation between the fall in GSH and that in acid phosphatase activity in both maternal and fetal RBC incubated with APH. It is also evident that for a given per cent fall in GSH level the acid phosphatase activity decreased more in fetal than in maternal RBC.

DISCUSSION

The acid phosphatase of human RBC presents a genetic polymorphism with 6 different electrophoretic phenotypes (6, 7) which show a different sensitivity to the action of oxidizing substances (12). We did not determine the electrophoretic phenotypes of RBC acid phosphatase in our cases and therefore we cannot be certain that the phenotypic distribution of maternal bloods was perfectly comparable to

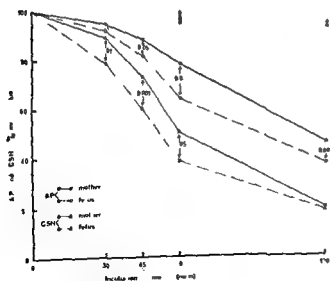


Fig 1 Changes in acid phosphatase activity (AP) and GSH concentration in maternal (● ▲) and fetal (○ △) RBC incubated for 2 hours at 37°C with and without APH. The significance of the difference at various intervals is also shown.

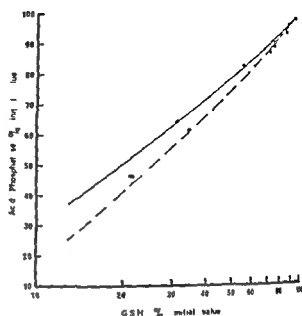


Fig 2 Relationship between the changes of GSH level and those of acid phosphatase activity (AP) in maternal (●) and fetal (○) RBC incubated with APH. Regression equations: Maternal RBC: $AP\% = 1.2 \times GSH\% - 69.0 - 39.6$, $r = 0.96$; Fetal RBC: $AP\% = 1.2 \times GSH\% - 84.2 - 68.2$, $r = 0.97$. The slopes of the two regression lines are significantly different ($p < 0.001$).

that of fetal and neonatal bloods. However, essentially the same differences between maternal and fetal blood were previously obtained in another laboratory under slightly different experimental conditions (5). Furthermore, since the fetal genotype is half determined by the maternal genotype, it seems unlikely that the phenotypic distribution of fetal bloods was very different from that of maternal bloods.

Other authors have demonstrated a significant GSH instability in the RBC of newborn infants. This is due in part to the physiological hypoglycemia and in part to the metabolic features of the neonatal RBC (11, 17). It is possible that GSH instability plays a role in the increased erythrocyte destruction which seems to take place during the first few days of life. However, at the present time this remains only a possibility and a speculation, since the biochemical events immediately preceding RBC destruction are not known. Our previous observations on the modifications induced by GSSG on RBC acid phosphatase (2-4) suggest

that the formation of mixed disulfides with GSSG could induce an inactivation of some enzymes and perhaps a modification of other structures in the RBC which could be important for the normal erythrocyte survival

The role of acid phosphatase in RBC metabolism is not known but it is possible that modifications similar to those observed by us for this enzyme could also be induced in other more important SH dependent structures

The results of the present study indicate that inactivation of acid phosphatase in RBC incubated with APH (without glucose) is preceded by a fall in GSH and that the two events are significantly correlated. It is therefore possible that the more rapid and marked fall in acid phosphatase activity of fetal RBC is the consequence of the increased GSH instability in these RBC when compared to the maternal erythrocytes. The GSH content of fetal and neonatal blood cells is higher than that of the adult (3, 14) therefore when the same percentage of initial GSH is oxidized more GSSG is formed in the fetal than in the adult RBC. This finding could explain why for a given percent fall of GSH in the RBC more acid phosphatase is inactivated in fetal than in maternal erythrocytes.

The uptake of APH by fetal and maternal RBC was not measured in the present experiments. Therefore the possibility that the observed differences between fetal and maternal RBC could be due at least in part to a different uptake of APH cannot be excluded.

The increase in acid phosphatase stability from birth to 3 days of age suggests that the fetal RBC population is not homogeneous and that those RBC which are less capable of protecting their SH dependent structures from oxidation are destroyed soon after birth. Some modifications of the internal milieu taking place early in extrauterine life such as a certain degree of hypoglycemia and an increased oxygen tension could favor this destruction and could contribute to the selection of a RBC population which is more stable in the changing biochemical environment.

SUMMARY

Incubation of red blood cells (RBC) *in vitro* with acetylphenylhydrazine and without glucose produced an inactivation of acid phosphatase which was more marked in the fetal RBC than in the RBC from maternal blood. The fall in reduced glutathione (GSH) during incubation was more rapid in fetal than in maternal RBC. In each instance the fall in GSH preceded that in acid phosphatase and the two phenomena were very well correlated. The faster inactivation of acid phosphatase in the fetal RBC could be due to the higher total concentration of GSH and to its decreased stability in fetal RBC.

The stability of acid phosphatase seemed to increase in red blood cells from infants 3 days old. This finding could indicate a certain degree of heterogeneity in the fetal RBC population at birth and a rapid selection in favor of those RBC which are more fit for the new biochemical environment after birth.

ACKNOWLEDGEMENTS

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A NEW SYNDROME OF DYSMORPHOGENESIS IMPERFORATE ANUS ASSOCIATED WITH POLY OLIGODACTYLY AND SKELETAL (MAINLY VERTEBRAL) ANOMALIES

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Although imperforate anus is commonly associated with congenital malformations of the genitourinary and gastrointestinal systems skeletal anomalies including club foot dislocation of the hips and sacral deformities are also frequently seen (2, 4, 8). In 1968 a new syndrome of dysmorphogenesis was described in which certain skeletal malformations including polydactyly were associated with imperforate anus (5). In this preliminary report ten unrelated patients were presented. Malformations of the vertebrae and the ribs were quite common in these patients while in some of them the iliac bones and the lower extremities were also involved.

The similarity of these findings to those seen among the members of the luxoid group of mouse mutants especially such as the dominant hemimelia (Dh mutant) (6) was quite striking hence the possibility of a hereditary factor as a cause of this syndrome was considered. Since these patients were ascertained through their polydactyly it was suggested that this syndrome may very well include forms of limb deformities other than polydactyly.

During a retrospective study of 258 cases of imperforate anus seen at this hospital between 1957 and 1969 (inclusive) nine patients were found to have hand malformations. This re-

port describes the malformations encountered in these patients together with a review of the similar cases in the literature.

CASE REPORTS

Case 1 O 4 4-day-old male was the second child of young parents. Physical examination additionally revealed polydactyly of the left hand and omphalocele. No X rays of the spine were taken. The father was 44 and the mother 23 years of age. There was no consanguinity between the parents and no family history of congenital anomalies including polydactyly.

Case 2 O 11 A 2-day-old male had an extra digit at right. X rays of the spine revealed the vertebrae to be normal. The left iliac bone was hypoplastic. No information regarding the family history was available except that the parents were not related.

Case 3 S 5 This 5-day-old female had bilateral postaxial polydactyly of the hands and the feet. She also had a butterfly vertebrae at Th6 and congenital heart disease the type of which is yet to be determined. The father was 26 and mother 24 years of age and they were not related. According to the parents there was no one in the family with congenital malformations.

Case 4 M 1 3-day-old male had an extra digit at left. He was the second child of this family. The father was 46 and the mother 19 years of age. Consanguinity between the parents and congenital malformations in the family were denied. On admission he was noted to have a tracheoesophageal fistula with oesophageal atresia in addition to the facial asymmetry and the absence of the right ear. X ray examination showed fusion of the bodies of Th6-

Table 1 General survey of the cases described

Case	Sex	Type of polydactyly	Vertebral anomalies	Parents age		Consanguinity	Other anomalies
				Mother	Father		
1	♂	Unilateral (Le) ^a	0	23	24	— ^b	Omphalocele
2	♂	Unilateral (R) ^c	None	20	23	0 ^d	Iliac hypoplasia (Le)
3	♀	Ulnar bilat	Butterfly vertebrae Th ₆	24	26	—	Cong heart disease (?)
4	♂	Fibular bilat	Fusion of the bodies of Th ₆ -Th ₇ hemivertebrae Th ₇ -Th ₈ and Th ₈ -L ₁	19	26	—	Tracheo-esophageal fistula with esophageal atresia facial asymmetry absence of the right ear
5	♂	Radial (Le)		0	0	—	Facial asymmetry malformed ear (R) iliac hypoplasia (Le)
6	♀	Radial (R)	0	22	30	—	—
7	♂	Radial (R)	Extra thoracic vertebrae	0	0	+	Syndactyly (left hand and left foot)
8	♂	Tibial Bilat	Distal sacral agenesis	30	36	0	Right renal agenesis left undescended testes
9	♂	Triphalangar thumbs bilat		19	24	—	—
10	♂	Radial (R)	None	19	24	—	—

^a Le - Left^b — - Absent^c R - Right^d 0 - Not recorded

Th9 and hemivertebrae involving Th2-Th6 and Th9-L1

Case 5 L A A 2 day old male was found to have extra thumbs bilaterally. He also had facial asymmetry and a malformed ear. X ray examination revealed left iliac hypoplasia and thoracic hemivertebrae. The parents were not related and there was no history of congenital malformations in the family.

Case 6 O M A 20-day old female had a supernumerary right thumb. The parents were not related. The father was 30 and mother 22 years of age. Unfortunately no X rays of the spine were taken. The parents denied the existence of similar or other congenital malformations.

Case 7 U 2 day old male had an extra thumb at right associated with bilateral preaxial polydactyly of the feet. In addition he had syndactyly between the 3rd and 4th left fingers and also between the extra digit and the first left toe. Only a part of the spine was seen in his X ray films which showed an extra thoracic vertebrae. No further information was available about the family except that the patient was the second child of this consanguineous family.

Case 8 I M S A 1 1/2-month old male who had triphalangar thumbs bilaterally. The X rays of spine revealed distal sacral agenesis. IVP revealed right renal agenesis. He also had undescended testes at left. No information with regard to the family history was available.

Case 9 A A 3 day old male infant who had an extra thumb at right. Skeletal X rays of the spine revealed no abnormalities. He was the first child of unrelated young parents (father 24 mother 19 years of age). The family denied any history of congenital malformations on either side of the family.

DISCUSSION

It is well known that congenital malformations may be multiple and that discovery of one should spur the search for others in the same patient. Many such combinations of anomalies have been collected into recognized syndromes. The purpose of establishing such syndromes is threefold. Firstly, they stress that seemingly different malformations are correlated and should be considered as a unit. Secondly, it stimulates a search for further components of the syndrome and finally it may lead to the discovery of the etiology through analysis of similar cases and hence perhaps may result in prevention when the pathogenesis is better appreciated.

Many frequent combinations of malformations other than those known at present probably exist and remain unrecognized. They may be infrequent enough to escape recognition or subtle enough to be missed on a cursory examination or of such a minor nature that little attention is focused on them. The optimal procedure for delineation of a new syndrome is undoubtedly the one based on an etiology. On the other hand when this is not possible one can collect a large number of patients with

Table 2 Hand malformations (25 cases)

	Preaxial	Postaxial	Unknown	Total
Polydactyly	12	2	3	17
Hypoplastic thumbs	3	—	—	3
Absence of the thumbs	3	—	—	3
Abnormality of the thumbs*	1	—	—	1
Triphalangeal thumbs	1	—	—	1
	20	2	3	25

* 2 further cases had polydactyly of the toes only
 † Not defined

are fully investigated precisely documented malformations and search for recurring similarities within this group. However in the absence of such large prospective studies investigation of sporadic cases or of their hospital records retrospectively will have to suffice although it should be remembered that a certain constellation of malformations may not necessarily indicate etiological uniformity. Publication of such patients we believe may alert other investigators to look for additional cases and hopefully to publish them leading to a comprehensive delineation of the syndrome in time.

This newly proposed syndrome of multiple malformations may actually constitute a good example for the potential importance of retrospective studies. Since the publication of the first report on this subject several observations have appeared in the literature which helped greatly towards a better understanding of the proposed syndrome (1, 3, 9).

In Table 1 the malformations seen in our cases with imperforate anus have been presented. Here the type of anorectal anomalies observed in individual cases have not been recorded. As it is known imperforate anus is a descriptive term and covers a heterogeneous group of malformations varying from minor deformities to complex anorectal anomalies. Among our patients there were 3 cases with anal stenosis (Ladd and Gross classification type 1) (2), one with an anal membrane (type

Table 3 Vertebral anomalies in 16 cases

Hemivertebrae	7
Neural arch anomalies	7
Fused vertebrae	5
Increased number of vertebrae	5
Bifid vertebrae	3
Other anomalies	7

II) and another one with type IV deformity. In the remaining 4 cases the anus was absent and the rectum was ending blindly (Type III) which is the commonest type of malformation encountered in clinical practice.

The various malformations outside the anorectal anomalies encountered among the 18 published cases with this entity together with those seen in our 9 patients are presented in Tables 2-4. Interestingly in 17 patients of a total of 25 (the remaining 2 patients had polydactyly of the toes only) the hand malformation was in the form of polydactyly while 3 patients had ectrodactyly, three had hypoplasia and one a triphalangeal thumb. These indicate that contrary to the observations in mice (10) will be discussed later polydactyly is seen more often than other forms of hand malformations even if one removes the 10 cases reported in the initial communication since they were ascertained through their polydactyly. It should be recorded however that of the six cases reported by Tunte (9) none had an extra digit while among our cases ascertained similarly through their anal malformations 11 patients out of 11 had polydactyly.

Table 4 Other malformations

	No. of cases
Tracheo-oesophageal fistula	3
Agensis of the kidney	2
Facial asymmetry	2
Malformed ears	2
Congenital heart disease	1
Agensis of the lung	1
Hypospadias	1
Omphalocele	1
Agensis of the spleen	1

There were 3 other cases with probable congenital heart disease.

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5	♂	Radial bilat	Thoracic hemivertebrae	0	0	—	Facial asymmetry mal- formed ear (R) iliac hypoplasia (Le)
6	♀	Radial (R)	0	22	30	—	—
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is not affected. In most of them the antero-ventral part of the left kidney is flattened and in the remaining it is hydropic. The stomach is smaller than normal in many with a reduced caecum in some. On the other hand in the Dh/Dh mice the stomach is much smaller and hydropic kidneys are usually seen in all. There is no anus in 44% of them and similarly a gap in colon or rectum or a blind ending rectum is seen in the same percentage of these mice. Reduction in the number of thoracic ribs and presacral vertebrae is seen in both Dh/Dh and Dh/Dh mutant mice although the malformations are much more severe among the latter (6).

The similarity between the findings observed in patients with this newly syndrome and in mice with dominant hemimegalia is indeed striking. Certain differences however should be mentioned. First of all the malformations involve generally the hind limbs in mice while the hand malformations seem to be more commonly encountered in man. Secondly anal atresia is usually seen in the homozygote mutant mice who rarely survive more than a few days and in whom always oligodactyly rather than polydactyly is seen. Finally dominant pattern of inheritance as in mutant mice has not been proven in man as yet. In fact the role of hereditary factors in the etiology of this triad is uncertain. So far with a possible exception of the family described by Fuhrmann (1) no evidence in this regard is available. Fuhrmann described a family in which a 6-week old boy had an imperforate anus and his older brother had the same anomaly in addition to unilateral radial polydactyly. Interestingly the mother herself had a unilateral polydactyly (1). In our series however no similar malformations existed among the members of the seven families where detailed family history could be obtained.

Similarly reports by others also failed to result in a positive family history. It may be argued that some of these cases may be the result of a new mutation although all could not be considered as such. It may be also spe-

culated that the negative family history is the result of several factors such as low penetrance of presumed mutant gene, decreased reproductive capacity and/or the short life span and hence the decreased fertility of the affected etc. All these probably indicate that the possibility of a dominant heredity can not be excluded at present.

The possibility of a chromosome disorder being the cause of this condition could not definitely be eliminated since chromosome studies have not been done in many of these cases. However the available observations although admittedly limited give no indication as to the presence of a distinct chromosome aberration which might be responsible for this condition. Environmental factors may also be responsible for this syndrome. Although family histories failed to reveal any teratogenic agent this possibility can not at present be definitely ruled out. All these indicate that further studies are in order to clarify this matter.

The true incidence of this condition among the live born infants is yet to be established. Among the 185 caucasian patients with polydactyly this combination of malformations was observed in 10 cases (5.4%) (5). However the incidence figure obtained from the above mentioned study due to the method of a certainment used may only represent a large referral hospital population. It is quite conceivable for instance that some of the patients with the milder form of polydactyly such as those with ulnar skin tags were treated at birth by their family physician and therefore would not be included in this particular study. It should also be recorded that many of these cases with polydactyly (34%) had other major malformations which were the main reason for their hospitalization (5). All these indicate that the prevalence of this syndrome among the patients with polydactyly is in fact considerably below 5%. Retrospective studies of the hospital records are expected to provide more reliable incidence figures for patients with imperforate anus since this latter condition will practically always necessitate hospitalization.

One common finding to the reported cases seems to be the site of the hand malformation. This was found to be preaxial in 20 instances out of 25 where it was recorded. On two occasions the extra digits involved the feet only, but again the location was preaxial in one of them (Table 1).

Vertebral anomalies of various types and severity were present in 16 cases out of the total 21 patients in whom the X rays of the spine were available for examination. In some patients the abnormality merely consisted of an increase in the number of the vertebrae while in others it was of more serious nature such as fusion of the vertebrae and hemivertebrae involving one or more vertebral bodies (Table 3). It is interesting that these deformities were presacral in 14 cases. Although as stressed by Williams & Nixon (10) various forms of sacral deformities including sacral agenesis are not too rare in patients with imperforate anus usually few vertebral abnormalities are encountered above the sacral level in these patients.

Malformations of the ribs were also relatively common among these patients. They were found in 8 patients. The types of malformations encountered again varied from patient to patient however hypoplasia and fusion of the costal bones as well as an increase in the number of them were among the more frequent abnormalities. It may be that rib anomalies are more common in these patients than the observations so far indicate since at least in our series satisfactory X rays were not available in most of the cases. However in some of the cases the malformations of the ribs were probably secondary to the vertebral deformities.

Another interesting finding was hypoplasia of the ilium which was found twice in our series and again on three occasions in the initial report by Say & Gerald (5). These peculiarly involved the left side on all these occasions. This predilection for one side has been noted in other deformities and its cause is still unknown.

Among the remaining associated malformations tracheoesophageal fistula with esophageal atresia was seen on three occasions (Table 4). The diagnosis of congenital heart disease was made in 4 cases. Although in the initial report on this subject no such case was encountered others subsequently published cases who had congenital heart disease in addition to the cardinal malformations. In dominant hemimelia mutant mice this malformation does not usually occur. However, in all these patients with the exception of the case reported by Tunte (9) the diagnosis of congenital heart disease was not definite and this possibility was raised because of the presence of a murmur. It seems that more data are necessary before one reaches a final decision in this matter.

Other infrequent malformations seen are also listed in Table 4. These include omphalocele, facial asymmetry, malformations of the ears, agenesis of the kidney and undescended testes among others. Rectovaginal, rectovesical and rectourethral fistulas were present in 6 cases. This is an expected finding and should probably not be considered separately from the anal malformations.

Dominant hemimelia mutants are a member of the luxoid group of mouse mutants. This mutant was discovered in 1954 by Carter in Edinburgh (6). The extremity abnormalities in the heterozygous mice (Dh/+) are confined to the preaxial site of the hind limbs. The expression of it is quite variable since it is no more than a slight thickening and lengthening of the hallux in some offspring while in others it is in the form of a triphalangeal hallux or an extra digit on that side as well as syndactyly between certain digits. In some others deficiencies are observed such as a loss of a digit or two or part of digit and even luxation and reduction in the length of one or both hind limbs. In the Dh homozygotes (Dh/Dh) the hind legs are short and twisted and usually show oligodactyly. The fore limbs reveal no abnormalities. These mutant mice also have many visceral abnormalities. Dh/+ mice lack spleen while the rest of the lymphatic system

is not affected. In most of them the antero-ventral part of the left kidney is flattened and in the remaining it is hydropic. The stomach is smaller than normal in many with a reduced caecum in some. On the other hand in the Dh/Dh mice the stomach is much smaller and hydropic kidneys are usually seen in all. There is no anus in 44% of them and similarly a gap in colon or rectum or a blind ending rectum is seen in the same percentage of these mice. Reduction in the number of thoracic ribs and presacral vertebrae is seen in both Dh/ and Dh/Dh mutant mice although the malformations are much more severe among the latter (6).

The similarity between the findings observed in patients with this newly syndrome and in mice with dominant hemimelia is indeed striking. Certain differences however should be mentioned. First of all the malformations involve generally the hind limbs in mice while the hand malformations seem to be more commonly encountered in man. Secondly anal atresia is usually seen in the homozygous mutant mice who rarely survive more than a few days and in whom always oligodactyly rather than polydactyly is seen. Finally dominant pattern of inheritance as in mutant mice has not been proven in man as yet. In fact the role of hereditary factors in the etiology of this triad is uncertain. So far with a possible exception of the family described by Fuhrmann (1) no evidence in this regard is available. Fuhrmann described a family in which a 6 week old boy had an imperforate anus and his older brother had the same anomaly in addition to unilateral radial polydactyly. Interestingly the mother herself had a unilateral polydactyly (1). In our series however no similar malformations existed among the members of the seven families where detailed family history could be obtained.

Similarly reports by others also failed to result in a positive family history. It may be argued that some of these cases may be the result of a new mutation although all could not be considered as such. It may be also spe-

culated that the negative family history is the result of several factors such as low penetrance of presumed mutant gene, decreased reproductive capacity and/or the short life span and hence the decreased fertility of the affected etc. All these probably indicate that the possibility of a dominant heredity can not be excluded at present.

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At this institution nine cases, presumably representing this syndrome were encountered among 258 cases with imperforate anus (3.4%). It is interesting that a similar figure was found in other series. Tunte (9) reported 6 patients with hand malformations among 103 imperforate anus cases and in a report by Stevenson et al (7) on the occurrence and type of congenital malformations encountered in 24 centers in 16 countries we could find 5 possible examples of this syndrome among 110 cases with imperforate anus (4.5%). From these figures an estimate of the incidence of this condition among the live born infants can be made. Since the incidence of imperforate anus is variously reported to be one in 1 000 to 5 000 caucasian live births (roughly 1/3 000) and since this syndrome is encountered in about 3 to 5% of the cases with imperforate anus, then the incidence of this condition among the caucasian liveborn infants should be one on 20 000 to 150 000 (1/75 000).

Finally it should be mentioned that in this communication patients having imperforate anus and hand malformations with or without vertebral anomalies have been considered. There were 5 patients in whom no abnormalities related to the spine could be found. The reason for their inclusion is the fact that in many well established syndromes some of the cardinal features may be missing. It is hoped that the publication of further new observations will bring more light to the understanding of this syndrome of dysmorphogenesis.

SUMMARY

Nine patients having imperforate anus and hand malformations with or without vertebral anomalies have been presented. The similarity of the findings in these patients to those seen among the members of the luxoid group of

mouse mutants such as the dominant hemimelia (Dh) mutants has been emphasized. Similar cases reported in the literature have been briefly reviewed.

ACKNOWLEDGEMENTS

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PURPURA AND ACETYLSALICYLIC ACID THERAPY

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The occurrence of purpura in a child often faces the paediatrician with the problem of diagnosing a disturbance with an ill defined etiology or a disease with bad prognosis. Fortunately this is not always the case and it was a relieving experience to find that in three of our patients with purpura this symptom was most probably caused by the ingestion of a normal dose of acetylsalicylic acid.

Purpura as a complication of acetylsalicylic acid therapy is not unexpected in view of the findings of Evans et al (4) Weiss et al (20) and O'Brien (11, 12) who independently of each other observed in 1968 that acetylsalicylic acid affected the "in vivo" and "in vitro" aggregation of blood platelets mainly by an inhibition of the release of adenosine 5'-di-phosphate (ADP).

Our case reports are as far as we know the first clinical descriptions of a purpura due to this mechanism.

CASE REPORTS

Case 1

A 10-month-old female infant was referred to the hospital because of purpura. She is the first child of healthy and unrelated parents. There is no history of familial haemorrhagic disease. The baby was born 6 weeks before term, birthweight was 2700 g. Physical and psychomotor development were normal. She always received a properly balanced diet. Two days before admission she developed a mild cough for

which she was given on three consecutive nights one suppository containing 65 mg of acetylsalicylic acid. On the morning of admission she developed purpuric spots on her face. The physical examination on admission revealed a normally developed child with a weight of 8750 g (P25) and a height of 70 cm (P25). The temperature on admission was 38.6°C, the heart rate 150/min and the blood pressure 90/50 mmHg. She did not look very ill and was not pale at all but she presented numerous petechiae. These were pin point sized, multiple and very densely spread over the face and neck but scarcely on the arms and trunk. There was neither bruising nor erythematous rash nor urticarial wheals. The Rumpel-Leede test was positive. The further physical findings were normal except for a purulent rhinopharyngitis. Liver and spleen were not palpable. A treatment with ampicillin and nosedrops was started. Routine blood and urine analyses gave normal values. The platelet count was 556 000/mm³. Nose and throat culture yielded no pathogens. Five days after admission all petechiae had disappeared and since then the baby remains well.

Case 2 and 3

A mother presented at the outpatients department with her two children both girls respectively 9 years and 14 months old because of purpuric spots.

We were told that the younger child presented this phenomenon for the second time. The mother made the remark that on both occasions the baby was teething and for this reason was given acetylsalicylic acid as an analgesic. The girl of 9 had received this drug because of some fever. Both children received approximately 30 mg/kg/day for 4 days. Their parents are healthy and unrelated, they have three children all of them in good health. There is no family history of bleeding tendency but it is noteworthy that the father on two occasions with an interval of 3 years had a severe gastric bleeding.

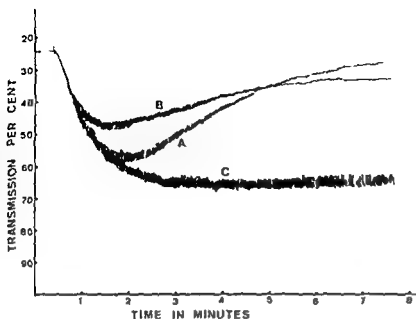


Fig 1 Platelet aggregation by ADP
A case 1 (first observation) B case 1
(second observation) C control

after a single intake of respectively 2 g and 1 g acetylsalicylic acid

On clinical examination both children looked healthy. Physical and psychomotor development were normal. The younger child had multiple petechiae roundshaped with a diameter of half to one mm in the neck and at the upper trunk. The older girl only had a few identical petechiae about ten spread over the anterior part of the neck.

The rest of the clinical findings were all normal. Routine blood and urine analysis also gave normal values. The platelet count was 230 000/mm³.

After exclusion of the most obvious causes it was assumed that in these three patients the purpura might be due to a defect in the platelet aggregation following acetylsalicylic acid therapy. Unfortunately only two of the three patients were available for further study of the platelet function.

MATERIAL AND METHODS

The blood for the haemostasis tests and for study of the platelet function was obtained by venous puncture and collected in plastic tubes containing 1 part of a solution containing 3.13 g sodium citrate for 9 parts of blood. Platelet rich plasma (PRP) was prepared by centrifuging blood at 400 g for 10 min. Platelet poor plasma (PPP) was obtained by centrifuging platelet rich plasma at 6000 g for 10 min. These procedures were performed at room temperature. Adenosine 5 diphosphate (ADP) and adrenaline were supplied by Stago Solutions containing 20 µg/ml were prepared just before use. A preparation of phospholipids Thromboplastin (Ortho) was used without further dilution. Russell's viper venom (Stypven, Burroughs Wellcome) was used as a 1/100 000 dilution in isotonic saline. Calcium was added from a 0.05 M solution of CaCl₂.

The platelet counts were performed using blood diluting pipettes (Unopette, Becton Dickinson). Plate

let aggregation in PRP was followed with Born's photometric technique (1) using an EEL 401 Absorptiometer (Evans Electroselenium Ltd) with built in stirrer and water jacket for temperature control. The absorptiometer was connected to a pen recorder (Vitratron) for automatic registration of variations in transmitted light. All experiments were performed at 37°C. PRP was adjusted to 300 000 platelets/mm³ by diluting with PPP of the same subject. For the aggregation tests 0.2 ml of aggregating agent was added to 0.8 ml of PRP.

Prothrombin (factor II) and factor V were assayed according to Stormorken (17). The one stage prothrombin time was performed according to Quici (15) and the P and P test according to Owen & Ari (14). The plasma fibrinogen level in mg% was determined using the Fibrin Polymerization Time (FPT) test (18) and the thrombin time was measured as described by Vermeylen & Verstraete (19).

Clot retraction was evaluated visually in whole blood after 1 hour incubation at 37°C and the result was expressed arbitrarily as a value between 1 (absence of retraction) and 4+.

Partial thromboplastin time was determined according to Langdell et al (9) and the availability of platelet factor 3 (PF 3) according to Spaet & Cintoni (16).

RESULTS

Case 1

The aggregation of the platelets in the patient PRP was studied for the first time 5 days after interruption of the acetylsalicylic acid therapy. The platelet aggregation by adding ADP was normal but was followed by a rapid disaggregation (Fig 1). The effect of adrenaline on the platelet aggregation is shown in Fig 2.

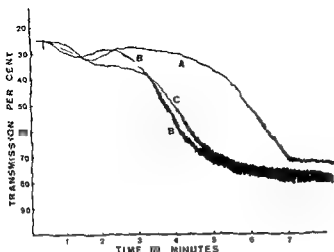


Fig 2 Platelet aggregation by adrenaline A case I (first observation) B case I (second observation) C control

Usually this aggregation occurs in two phases a first one which depends on adrenaline itself and a second one which depends on release of endogenous ADP. In our patient the first wave of aggregation was normal but the second one appeared with an appreciable delay.

As was shown by the Giactano et al (2) "Thrombofax" which contains lipid known as cephalins induces a strong aggregation after a latent period of 60–80 sec. This action of Thrombofax seems to be similar to that of collagen. The addition of thrombofax to PRP of our first patient induced aggregation only after a prolonged period of time (Fig 3). The availability of PF 3 was normal.

After 20 days during which period the patient did not receive acetylsalicylic acid all tests were repeated. As shown in Figs 1, 2 and 3 the alterations of the platelet function which were observed at the first investigation had completely disappeared.

The other haemostasis tests performed in this patient during and after acetylsalicylic acid therapy gave normal values with exception for the platelet counts which were rather high (Table 1).

Case 2

The aggregation tests performed on the PRP of this patient (the 9 years old girl) during

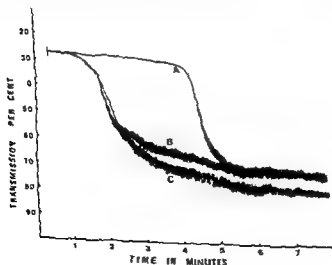


Fig 3 Platelet aggregation by phospholipids A case I (first observation) B case I (second observation) C control

Table 1 Results obtained for the various hemostasis tests performed in case 1 and 2 while under the influence of acetylsalicylic acid (A), and after interruption of the drug for more than 15 days (B)

	Normal values	Case 1		Case 2	
		A	B	A	B
Blood platelets/mm ³	140 000/300 000	556 000	500 000	230 000	260 000
Bleeding time	30 -/3	4.50	3	5.20	2.10
Clot retraction	++++	++++	++++	++++	++++
Prothrombin time					
(undiluted plasma Quick)	100	100	100*	100	100
(diluted plasma Owren)	100	100	■	100	75*
Factor V	100	100	100*	100	95
Prothrombin in plasma	100	100	100*	100	95
Thrombin time	18-24 sec	18 sec	20.4 sec	18.5 sec	21.5 sec
Fibrinogen (total blood)	90-210 mg	220 mg	222 mg	147 mg	95 mg*
Partial thromboplastin time					
(without activation)	100 sec	76 sec	92 sec	87 sec	65 sec
(with activation)	< 60 sec	40 sec	47 sec	28 sec	34 sec

acetylsalicylic acid therapy showed similar alterations as in case 1. However, the disturbances of the platelet function were more pronounced. Addition of ADP caused a normal aggregation, but a more rapid disaggregation ensued. The second wave of aggregation after addition of adrenaline was completely absent and the clumping induced by Thromboplastin was markedly reduced. All these alterations of the platelet function had completely disappeared at a second investigation three weeks after the interruption of the acetylsalicylic acid therapy.

As in case 1 the availability of PF 3 and the other hemostasis tests were normal (Table 1).

DISCUSSION

Purpura in a patient with normal platelet count can be due to a vascular defect usually described as vasculitis or to an abnormality of the platelet function.

Acetylsalicylic acid in a toxic dose has been reported to be responsible for purpura. Indeed petechial haemorrhages can be a prominent feature at post mortem examination of individuals dying from acetylsalicylic acid poisoning (7). Non thrombocytopenic purpura has also been observed in patients receiving a normal dose of acetylsalicylic acid but this distur-

bance was believed to be due to hypersensitivity (6).

In our patients overdosage as well as idiosyncrasy can be excluded. A single test dose of acetylsalicylic acid (5 mg/kg) given to our patients did not cause a reappearance of the symptoms.

Frick (5) reporting in 1956 on haemorrhagic diathesis caused by salicylate therapy stated that acetylsalicylic acid induced a positive Rumpel-Leede test and an abnormal bleeding time in his patients. To explain this phenomenon at that time he forwarded the hypothesis of an increased capillary fragility. However the recent investigations of the effect of acetylsalicylic acid on blood platelets suggest that also in these patients a disturbance of the platelet function rather than a vascular defect might be responsible for his findings.

The normalization of platelet function 20 days after interruption of the acetylsalicylic acid therapy provides evidence that the abnormal behaviour of platelets in our patients was due to the intake of acetylsalicylic acid and cannot be considered as a Portsmouth syndrome (10).

This effect of acetylsalicylic acid on platelet function was first observed in 1968 independently by Evans et al (4), Weiss et al (20)

and O'Brien (11, 12) and has been studied intensively during the last 2 years. Ingestion of acetylsalicylic acid causes an impaired aggregation of platelets mainly by inhibiting the release of endogenous ADP.

The *in vivo* release of ADP from stimulated platelets contributes to the adhesion of more platelets to those already stuck to a site of injury. Acetylsalicylic acid by interfering with this release inhibits this phase of aggregation and may in this way affect the hemostatic properties of platelets. Therefore it is not unexpected that it can induce purpura in a patient especially if an additional local factor provokes some capillary damage. The dense appearance of purpuric spots on the face and neck of our first patient might be due to the interaction of the disturbed platelet function and a local increase of pressure in the capillaries e.g. during coughing. A similar problem can be found in the haemorrhagic effect of acetylsalicylic acid in the gastro-intestinal tract. Such bleeding was first attributed to local gastro-intestinal irritation (3) but its occurrence after intravenous administration (8) made Weiss (20) suggest that this haemorrhagic action is partly due to impaired release of platelet ADP.

Noteworthy is the fact that the father of our patients described under case 2 and 3 suffered from a severe gastric bleeding on two occasions after a single intake of respectively 2 g and 1 g of acetylsalicylic acid. Two of his children developed purpura while taking the same drug. These phenomena can be independent of each other but they could as well be due to some familial predisposition to develop this impaired platelet function after ingestion of acetylsalicylic acid. The question arises whether this predisposition might not explain the rather low frequency of these clinical complications.

The alterations of platelet function after a single ingestion of acetylsalicylic acid remain detectable four to seven days after the intake although the serum salicylate level is raised only for a few hours after ingestion of the drug.

This fact suggests that platelets and presumably megakaryocytes are specifically and irreversibly modified by acetylsalicylic acid and that only after 4 to 7 days sufficient normal platelets reappear in the circulating blood thus normalizing the platelet aggregation tests (12).

It is also important to stress that many of the compounds sharing one or more of the pharmacological properties of acetylsalicylic acid exert an inhibitory effect on the platelet aggregation (13). The following drugs were found to have this inhibitory action: *in vitro* meclofenamic acid, acetylsalicylic acid, indomethacin (Indocid), ibuprofen, mefenamic acid (Ponstan), amidopyrine (Pyramidon), dextro-propoxyphene hydrochloride (Doloxene), paracetamol, ibufenac (Dytransin) and phenylbutazone (Butazolidin). In contrast oxyphenbutazone (Tanderil), phenacetin, gentisic acid, codeine, dihydrocodeine and sodium salicylate did not have this inhibitory effect.

From our clinical observations and these studies we would like to draw two conclusions. Firstly that the possibility of a platelet dysfunction due to an inhibition of ADP release following acetylsalicylic acid therapy should always be considered in the differential diagnosis of a non thrombocytopenic purpura. Secondly it would be safer not to use acetylsalicylic acid and the other anti-inflammatory compounds mentioned above in children with bleeding tendency as for instance in the thrombopenic, the leucemic and the hemophilic child.

SUMMARY

Three cases are reported of children presenting purpura while taking a normal dose of acetylsalicylic acid since a few days. Studies of the platelet function showed an impaired platelet aggregation by an inhibition of ADP release. It is concluded that this mechanism is responsible for the clinical findings and that an acetylsalicylic acid therapy should always be considered in the differential diagnosis of a non thrombocytopenic purpura.

Table 1 Results obtained for the various hemostasis tests performed in case 1 and 2 while under the influence of acetylsalicylic acid (A), and after interruption of the drug for more than 15 days (B)

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CHRONIC REGIONAL ENTEROCOLITIS (MB CROHN) IN CHILDREN AND ADOLESCENTS

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During the last decade a marked increase in the occurrence of Regional Enterocolitis in adults has been observed in Sweden (10). In our institution a corresponding increase was noted in the pediatric age group (including children and adolescents under 16 years). Prior to 1959 isolated cases only were seen while a regular annual occurrence of 2-5 cases has been noted during the last decade. This development is similar to that reported previously with reference to Ulcerative Colitis in children (11). So far little attention has been paid to the specific pattern of the disease in children (1, 6, 8, 15, 18, 21). A report of our experiences in diagnosis and treatment of a 10-year series therefore seems timely.

MATERIAL

36 patients with Regional Enterocolitis were treated from 1959 to and inclusive of 1969. 24 boys and 12 girls. The majority 31 have been subjected to surgical therapy. The age distribution at onset and at operation is shown in Table 1. Most patients were above 9 years of age at onset. The diagnosis has been confirmed by microscopy of the resected specimens showing the typical picture of granulomatous enterocolitis. In the 5 cases not operated upon the diagnosis was based on typical clinical and radiological findings.

CLINICAL FINDINGS

Symptoms and signs. In our series an insidious onset was very common and was often misinterpreted in 13 patients more than 2 years

elapsed from the appearance of the first symptoms to the establishment of the diagnosis. In 7 patients the disease remained undiagnosed for more than 4 years. Non specific symptoms fatigue anorexia weight loss and retardation of growth were of common occurrence. In some patients retardation of puberty was evident (Fig. 1). General symptoms sometimes preceded the local manifestations for a long period of time. The most common local symptoms were abdominal pains (82%) and diarrhoea (88%). Diarrhoea was as a rule less severe than in Ulcerative Colitis. Only one third of the patients had bloody stools. 9 patients had perianal fistula and/or abscess formation. 3 of them as a presenting symptom. A palpable mass in the abdomen was encountered only in 4 cases. Intraabdominal fistulation occurred in 2 patients. In 1 case to the bladder. 7 patients had extraintestinal manifestations (arthritis erythema nodosum or liver damage).

The following case report illustrates a common type of clinical course. Boy born 1950 eczema in infancy 1957 prolonged fatigue and poor appetite after family infection with influenza. Anemia and high sedimentation rate found at repeat examinations by school physicians and in out patient clinics no diagnosis. Intermittent diarrhoea and abdominal pain since 1961. Barium enema and intestinal series 1961 showed typical picture of Crohn's disease of terminal ileum and proximal colon (Fig. 2). 1961-1964 intermittent severe gonitis treated with exsufflation and local injections of prednisolone. Patient also treated with salicylazosulphapyridine high caloric protein rich diet vitamins and (for shorter periods) with prednisolone.

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Table 2 Localization

Localization	No. of patients
Small intestine only	4
Colon only	2
Small intestine and colon	11
Colon and rectum	1
Small intestine and colon and rectum	4
Total	31

the terminal ileum with irregularly contoured mucosa and stenosed lumen. The localization of the disease is shown in Table 2. 27 of the 31 patients had changes in the colon, most of them with the small intestine engaged as well. Skip areas, cobble stone pattern and pronounced strictures were frequent findings. The earliest sign of colon changes was segmentally increased haustration. Later on dehausturation and often transverse mucosal ulcerations could be seen.

Table 3 Common and distinctive features in ulcerative colitis and regional enterocolitis

	Ulcerative colitis	Regional enterocolitis
Localization		
Small intestine	- (except backwash ileitis)	+ + -
Colon	-	+ +
Rectum	++	+
Skip areas	-	+ +
Pathology		
Strictures	-	++
Abscess & fistula formation	-	+ +
Perforation, diffuse peritonitis	-	-
Cancer	+ +	-
Granulomatous inflammation	-	++
Giant cells	-	++
History		
Marked anorexia	-	++
Abdominal pains	-	++
Blood in stools	++ +	+
Extraintestinal manifestations	++	+
Retardation	++	++ +
Remissions	++	-
Laboratory findings		
Sideropenic anemia	+ + -	- + +
B ₁₂ deficiency	-	-
Protein leakage	+ +	+

Table 4 Procedures performed at primary surgery

Procedure	No. of patients
Ileocecal resection	10
Resect of dist ileum and/or total colectomy + c.a.	8
Resect of dist ileum and hemicolectomy + c.a.	5
Resect of dist ileum + pancoloproctectomy + ileostomy	4
Separate resect of small and large intestine	3
Resect of dist ileum and colectomy + ileostomy	1
Total	31

Some of the radiological features are demonstrated in Fig. 2.

Eklöf & Gierup (7) studied the retrorectal soft tissue space in 160 normal children and in 28 children with Regional Enterocolitis, in 8 of whom the space was increased. The increase was generally less marked than in Ulcerative Colitis and did not aid materially in the differential diagnosis. At operation these cases showed a marked proctitis-periproctitis.

DIAGNOSIS

Preoperative diagnosis was based on clinical and radiological findings. In the early stages, absence of local symptoms and signs were a common cause of diagnostic difficulties, in particular in differentiating Regional Enterocolitis from Malabsorption, Collagen Disease and Psychological Disturbances. Later on, the differentiation of local symptoms from those in Ulcerative Colitis was the main diagnostic problem. A synopsis of similarities and differences between the two diseases has been compiled in Table 3. In regional Enterocolitis, abdominal pain and pronounced anorexia are often encountered. In Ulcerative Colitis, these symptoms are rare. Diarrhoea is usually less marked in Regional Enterocolitis and presence of blood is less common. Localization to the small intestine and the radiological characteristics listed above are as a rule decisive in distinguishing between the two diseases. Peri-

Table 1 Age at onset and at operation in regional enterocolitis

Age (years)	At onset	At operation
3-5	1	
6-8	4	
9-12	21	8
13-16	5	21
17-19		2
Total	31	31

Results of therapy unsatisfactory patient invalidized by arthritis moderate growth retardation

1965 operated with resection of diseased bowel. The specimen showed changes typical of granulomatous enterocolitis.

Uncomplicated post operative course quick restoration of appetite and strength weight gain 9 kg in 6 months normal pubertal development regression of joint symptoms hemoglobin and sedimentation rate normalized Normal school achievement later trained as clerk. 1969 deterioration of general state of health poor appetite weight loss slight joint symptoms sedimentation rate moderately elevated X-ray examination demonstrates relapse in colon reoperation planned.



Fig 1 Growth retardation in chronic regional enterocolitis. Patient G S (left) 13 years old symptoms approx 5 years. Control identical twin B S (right) healthy.



Fig 2 Typical X-ray picture in regional enterocolitis in terminal ileum caecum and ascending colon (courtesy by Dr U Rudhe Karolinska Sjukhuset)

LABORATORY FINDINGS

An increased sedimentation rate was found in all patients occasionally extreme values (>100 mm/1 hour) were noted. The majority of patients showed a sideropenic anemia and an increased number of blood platelets ($>400\,000/\text{mm}^3$). About half of the patients showed hypoproteinemia hypoalbuminemia or hypergammaglobulinemia. Intestinal protein leakage was demonstrated in 2 patients investigated in this respect.

RADIOLOGY

Barium enema and barium meal studies were performed in all patients as a rule repeatedly. The results in the first 15 cases have been reported previously (19). The most common finding was thickening of the intestinal wall in

few exceptions this occurred within the first 2 years postoperatively

An assessment of the radicality of the operation based upon re-examination of the X ray films, operative findings and microscopical findings of the resected specimen was performed (Table 8). In half of the cases (15) only the operation was judged to be radical. In 12 cases radicality was questionable and in four patients resection was incomplete. All of the patients in the last group had a recurrence. In the radically resected and in the questionable groups the recurrence rate was about 40.

8 recurrences out of 11 in the radically resected and questionable groups occurred in patients with wide spread disease. 4 out of 5 cases with ileorectal anastomosis had a recurrence. Three of these patients showed a thickening of the retro cecal tissue space, proctitis or perianal fistula prior to operation.

Among the 15 patients with recurrence after primary surgery 9 have so far been subjected to re-operation. In one the result was good. In four there was a new recurrence. In four the follow up time is less than 2 years. Two patients were operated upon a third time. In both there was again a recurrence.

DISCUSSION AND CONCLUSIONS

The increased incidence of Regional Enterocolitis in recent years seems in part to be explained by an increased awareness and more adequate diagnostic measures. As in Ulcerative Colitis however a true increase seems highly probable. The diagnostic difficulties encountered in the early stages of the disease, presenting with nonspecific symptoms, are evident. Weight loss, retardation of growth and puberty and increased sedimentation rate in patients with fatigue and anorexia should arouse suspicion of a chronic disease including the possibility of a diagnosis of Regional Enterocolitis. Barium meal and barium enema studies should be included in the routine examinations of such patients. In particular the importance of gastrointestinal X ray studies in patients with chronic

citating or recurrent perianal fistulas should be stressed.

Weight and sedimentation rate are good indicators of the activity of the disease. Abnormal findings postoperatively are indicative of a recurrence (23).

In recent years an increasing number of cases with colonic localization has been reported (2, 4, 13, 22). In large series the incidence of colonic disease is reported as varying between 10-50% (5, 10, 17). In our series 27 out of 31 cases treated surgically had changes in the colon. A high incidence of colon involvement thus would seem to be typical for the disease in children and adolescents. Mowley et al. (15) however found colonic changes in 4 patients only out of a total of 28.

Extraintestinal manifestations seem to be more common in Ulcerative Colitis than in Regional Enteritis. A comparison between our material gives the figures as 50 and 20 respectively.

Restraint with surgery has formerly been recommended in view of a postulated tendency to spontaneous remission and of the risk of postoperative recurrence (12, 17). In our series the results of conservative treatment were disappointing and did not prevent progression of the disease. Similar results were reported by Anfanger (1), Cornes & Stretcher (4) and Jones & Lennard Jones (9). We therefore felt justified in trying a more active surgical approach. As regards technique most surgeons nowadays consider a one stage radical resection of all diseased bowel to be the method of choice.

Recurrence rates following primary surgery of between 20 and 65% have been reported (3, 8, 14, 17, 23). It has been claimed that the recurrence rate is lower when the disease is located in the colon (4, 13, 20). In our own series with its high incidence of colonic localization the recurrence rate was about 50% indicating that colonic localization does not seem to offer protection from a high recurrence rate.

The importance of radicality is indicated by the fact that all of the 4 patients in our series

Table 5 *Length of follow up periods after primary surgery*

Time years	No of patients
<1	5
1-2	5
2-4	11
4	10
Total	31

anal abscess or fistula formation is strongly indicative of Regional Enterocolitis. Microscopical examination is however, sometimes necessary for the differentiation especially when the lesion is confined to the large bowel.

THERAPY

In the early part of this series prolonged intensive conservative treatment was favoured: administration of protein, iron, salicylazosulphapyridine, blood transfusions, vitamins and corticosteroids. The tendency to remission however, proved to be slight. With corticosteroids temporary improvement could be achieved but in general progression of the disease soon occurred. The adverse effects of the disease on weight, growth and puberty prolonged disability and inefficacy of conservative treatment encouraged us to a more active approach. Chronic progressive disease was the main indication for surgical treatment (23 patients). Severe arthritis was the indication in 2 cases. 6 emergency operations were performed three because of intestinal obstruction, two due to an acute fulminating type of disease and one on the assumption of appendicitis. The time elapsing between the first appearance of symptoms and surgery has been rather short—as an

Table 6 *Postoperative complications*

Type	No of patients
Intestinal obstruction	6
Leakage from anastomosis	2
Disruption of wound	2
Convulsions	1
Total	11

Table 7 *Results of primary surgery*

	Follow up >2 years	Follow-up <2 years	Total
No recurrence	8	8	16
Recurrence	13	2	15
Total	21	10	31

Table 8 *Assessment of radicality*

	Radical	Non radical	Questionable	Total
Recurrence	15 6	4 4	12 5	31 15

average 3 years. (In our material of Ulcerative Colitis the corresponding period was around 7 years.)

The principle of surgical treatment adopted has been resection of all diseased bowel where possible with restoration of intestinal continuity. The various surgical procedures used are listed in Table 4.

The length of follow up periods after primary surgery is shown in Table 5.

EARLY RESULTS

Neither operative nor late mortality has been encountered. The immediate results of surgery were good. All patients were relieved of pain; the diarrhoea diminished; appetite and mood improved. The average weight gain during the first 6 months postoperatively was 6 kilograms. Haemoglobin and sedimentation rate values normalized. Patients with ileostomy were well adapted and have recommended the operation to other patients.

Early postoperative complications causing temporary troubles only are listed in Table 6. Only two out of nine patients on steroid medication had such complications.

LATE RESULTS

Late results of primary surgery are shown in Table 7. 15 patients had a recurrence. With

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who in retrospect proved to have had non-radical resections performed developed recurrences (or, rather, continued disease). Similar findings have recently been reported by Wenckert et al (23).

A recurrence rate of 40% was, however, found even when radical resection has been performed. In our experience the extent of the lesion is an important prognostic factor in particular the high risk of recurrences after extensive resections of small bowel and/or colon with ileo rectal anastomosis should be stressed (13). In the case of extensive disease with rectal involvement leaving the rectum behind seems to be less favourable in Regional Enterocolitis than in Ulcerative Colitis. Before such operations are performed a careful investigation of retrorectal soft tissues, rectal mucosa and perianal manifestations should be performed.

In view of the smooth adaptation of children to ileostomy we have not hesitated to perform a pancoloproctectomy when indicated by the extension of the disease. So far we have not seen malabsorption develop postoperatively. In cases where extensive resections of the distal ileum has been performed vitamin B12 should be given prophylactically.

In order to decrease present high recurrence rates it has been suggested (10, 23) that resections should be more radical including segments of radiologically and macroscopically normal bowel in particular in colon resections. Postoperative treatment with salicylazosulphapyridine and/or corticosteroids has also been suggested as a possible prophylaxis against recurrences. None of these methods have however had an adequate trial so far.

An interesting approach has been reported by Oberhelman et al (16) achieving promising short term results by a temporary diverting ileostomy—a procedure which has been tried and abandoned in the treatment of Ulcerative Colitis.

The high incidence of recurrence makes surgery less encouraging in Regional Enterocolitis than in Ulcerative Colitis. On the other hand the progressive nature of the disease and the

poor effect of conservative treatment force us to continue the surgical defense until research has given us new weapons against this puzzling disease.

SUMMARY

A series of 36 children and adolescents with chronic regional enterocolitis is presented. An apparent increase in the occurrence of the disease is noted. Clinical and roentgenological findings are reported. A high incidence of colonic localization was found and is probably typical of a paediatric series. There were difficulties in establishing an early diagnosis mainly due to the common occurrence of non-specific symptoms in the early stages of the disease. The guiding principles for the differentiation between regional enterocolitis and ulcerative colitis are discussed. The results of conservative treatment were poor. The majority of patients (31) were subjected to surgical therapy, the main indication being chronic progressive disease. The immediate results of surgery were good. In respect of late results a recurrence rate of about 50% was encountered. Extensive disease especially when treated with resection and ileo rectal anastomosis seemed to have an unfavourable prognosis. The importance of radical resections is stressed.

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EPIDIDYMITIS IN CHILDREN

A Brief Review together with Reports of Six Cases

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The occurrence of acute epididymitis during the prepuberal period is considered a rare phenomenon. In patients in that age group, an acutely developing lesion of the scrotum will usually be torsion of the testis or incarcerated hernia. Certainly other diseases may also be responsible but as a rule they will not give rise to differential diagnostic doubts (1-6, 13).

Epididymitis in children is not mentioned in the current text books on paediatrics and data are scanty in the literature on the matter (2, 6, 13). Within the recent 4 year period we have seen 6 cases in the paediatric department in Glostrup Hospital, in two of these cases the development formed part of Schönlein-Henoch's purpura. This complication has been mentioned only twice before (7, 9) and publications have never included pictures of biopsies from epididymis which at microscopic examination presented the characteristic changes known from Schönlein-Henoch's purpura and other systemic diseases.

CASE REPORTS

Case 1

A 6 year old boy was hospitalized on the suspicion of an acute abdomen. Hitherto he had been healthy and in particular he had no allergic symptoms and was not either predisposed to allergy. On the day of admission pains reminding of abdominal colics set in. At the physical examination the abdomen felt soft to the touch although there was some diffuse tenderness. In the course of the first few days the condition aggravated and the boy discharged sang

unolent faeces. Microscopic examination revealed haematuria and the temperature was fluctuating. He went into shock and the condition did not improve until treatment by parenterally applied fluid was instituted. Five days after admission cutaneous haemorrhages typical of Schönlein-Henoch's purpura appeared, but neither oedema nor articular swelling occurred. Three days later reddening, swelling and tenderness of the scrotum set in together with turgidity of the scrotal content and swelling of the right funicle. On the suspicion of torsion of the testis an operation was performed at which marked oedema and swelling of the epididymis was found in particular at the site of the caput while the testis was of normal appearance. A biopsy taken from the caput revealed an inflammatory infiltration with many eosinophil cells and necrosis of the vascular wall whereas the ductules were well preserved. Symptoms disappeared within a few days and nothing except a moderate proteinuria and microscopically visible haematuria persisted at the time of discharge.

Four months later the boy was readmitted on account of abdominal pain and crural petechia. Four days after admission he had an episode of right-sided clonic spasms combined with corresponding left-sided EEG changes. Spasms had not previously been experienced and the episode should probably be taken as an influence on the brain involved in Schönlein-Henoch's purpura just like the persistent glomerulonephritis. It was not until 9 months after the first hospitalization that he became completely symptom-free and that all laboratory tests including tests of urine gave normal findings.

Laboratory tests SR 26 Leucocyte count 6300/mm and normal distribution. Thrombocyte count 500 000-1 000 000/mm throughout the first 4 months. No eosinophilia. AST 400 (elevated) normalized after 2 months. ASH normal findings. Culture of urine no growth and no leucocyturia. Parotitis complement fixation test negative. GO and GP transaminase lactic acid dehydrogenase serum electrophoresis blood urea and plasma creatinine normal findings. Emet. Weil and Widal tests negative. Coagulation time

bleeding time and prothrombin values normal Swabs from the throat showed growth of β haemolytic streptococci. Pathogenic intestinal bacteria were not in evidence nor were coxsackie virus echo- and poliovirus demonstrable in faeces Radiography of thorax and intravenous urography normal findings BP normal ECG normal findings

Case 2

A hitherto healthy boy of less than 6 years was hospitalized on the suspicion of an incarcerated inguinal hernia There was no history of allergy A few hours before admission he felt pain in the scrotum At the physical examination the right half part of the scrotum was found to be reddish and swollen the scrotal content had increased and the funicle had thickened At operation the epididymis was found to be swollen but the testis was normal A biopsy taken from cauda epididymis revealed changes similar to those observed in case 1 except that they were less marked Ten days after admission a haemorrhagic exanthema typical of Schönlein Henoch's purpura appeared on both legs Oedema of the ankle was negligible The patient had no medication during hospitalization and his temperature remained normal

Laboratory tests SR 17 AST and ASH normal findings Thrombocyte count 700-850 000/mm³ Microscopy of urine normal findings Culture of urine no growth Parotitis complement fixation test negative Intravenous urography revealed splitting of the left-sided renal pelvis otherwise normal findings Cysto-urethrography of micturition normal findings

Case 3

A boy aged 3 months was hospitalized on the suspicion of an incarcerated inguinal hernia Pregnancy and parturition had been normal Weight at birth 4 200 g No history of allergic diseases Since his birth there had been a tendency to regurgitation which aggravated when he at the age of 1 month was fed milk mixtures in stead of mother's milk growth and development were normal On the day of admission a tender massing occurred in the right half of the scrotum The temperature rose up to 38°C An operation was performed at which the epididymis was found to have thickened throughout cauda epididymis was the site of a solid infiltration the size of a hazelnut from which a biopsy was taken The testis was normal but the funicle had thickened and was oedematous The biopsy showed non specific inflammatory changes with few eosinophil cells and normal vessels but the epithelium of the ductules was damaged

Seven days after the operation the patient suddenly had several episodes of vomiting and his faeces were of a watery consistency Within a few hours he became dehydrated and went into shock but after parental application of fluid the condition improved A similar episode occurred 7 days later and like the first immediately upon intake of Eledon Since several provocative tests using mother's milk had released

identical reactions the condition was presumed to be allergy to milk for which reason the patient was fed soybean milk He did not either tolerate fruit which consequently was withheld from the diet It was not until 7 months after discharge from hospital that he came to tolerate milk and fruit At that time he was 1 year old he was of normal length but under weight by 3 kg

Laboratory tests SR 9 Leucocyte count 7 000/mm³ and normal distribution Eosinophil cell count which at admission had been 63 rose up to 695/mm³ after the first acute episode Thrombocyte count 930 000/mm³ determined at a time when the patient was satisfactorily hydrated values normalized however within 2 weeks Microscopy of urine normal findings Culture of urine no growth In addition the following tests showed normal conditions Parotitis complement fixation test fructose saccharose and lactose tolerance tests sweat test fractionated cortisol analysis serum electrophoresis GO and GP transaminase alkaline phosphatase galactose 1 phosphatase undiluted transference culture of blood and spinal fluid Urine was examined with a view to pregnantriol amino acid chromatography and owl-eye cells Faeces was examined for pathogenic intestinal bacteria and virus Intravenous urography was performed as well as radiography of the stomach and the intestinal passage

Case 4

A boy aged 2 months was hospitalized on the suspicion of left-sided orchitis Pregnancy and parturition had been normal Weight at birth 3 000 g He was breast fed for 3 weeks and subsequently fed milk mixtures His development was satisfactory and he was in good health until the day before hospitalization when he became unquiet and his temperature rose up to 38.2°C As the left half part of the scrotum was swollen and tender an operation was performed When the scrotum was opened large volumes of a tarnished fluid were evacuated Epididymis was found to the site of considerable inflammatory processes involving marked reddening oedema and moderate fibrin coating whereas the testis and the funicle were of normal appearance The biopsy obtained did not contain sufficient tissue for a diagnosis to be established by microscopy

Laboratory tests SR and leucocyte count normal findings Microscopy of urine normal findings Culture of urine no growth Intravenous urography normal findings Culture from the epididymis showed the presence of *Escherichia coli*

Case 5

A 5 week old boy was hospitalized to be observed for a left sided torsion of the testis Pregnancy and parturition had been normal Weight at birth 4 350 g He had been in good health until the day before hospitalization when he began to scream incessantly and his parents noticed a reddening and swelling of the scrotum The temperature was normal The physical examination revealed an increased content in the scrotum and a thickened funicle On the suspicion of

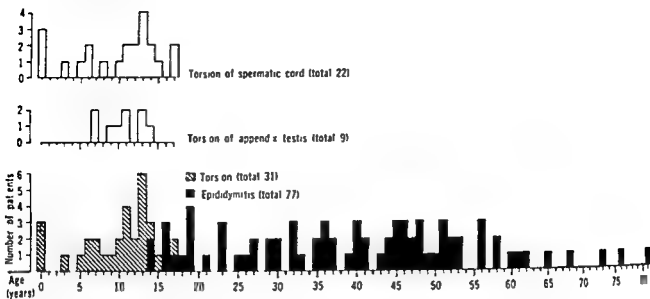


Fig. 1 Comparison of age distribution in patients with torsion of the spermatic cord torsion of appendix testis and epididymitis studied by Amar & Chhabra (1)

torsion of the testis in operation was performed at which the membranes were found to be oedematous cruda epididymis was the site of an infiltration the size of a hazelnut otherwise the epididymis and the testis were of normal appearance. The biopsy showed changes similar to those observed in case 3 although they were more marked.

Laboratory tests SR leucocyte count and differential count normal findings. No eosinophilia. Paratuberculous complement fixation test and Emu test negative. No evidence of coxsackie virus nor of echo and poliomyelitis virus in faeces. Microscopy of urine normal findings. Culture of urine no growth. Intravenous urography normal findings.

Case 6

The patient was a 5 year old boy in whom epididymitis developed after a right sided orchiopey because an infected nylon suture had penetrated the tip of caudal epididymis.

DISCUSSION

Reports concerning the incidence of epididymitis in children in the prepubertal age group vary greatly. During a 10 year period no more than 11 children with epididymitis were admitted to the Children's Hospital in Columbus, Ohio (6). The diagnoses were verified at operation or by positive cultures of urine. Among 610 patients examined by Mittenmeyer et al (12) only eight were children. Other authors who examined large series of patients did not find any cases of epididymitis among children. In a Danish

survey (8) of a series comprising 32 patients only one was a child.

The clinical diagnosis may be difficult to establish and often it will not be arrived at until an operation is performed. Usually the lesion will be regarded as torsion of the testis. Both lesions may be responsible of pain in the scrotum, reddening and oedema of the skin together with turgidity and tenderness of the scrotal content. It applies as a general rule that torsion of the testis will be associated with severe pain but normal temperature which is in contrast with epididymitis. In childhood however, symptoms may be deceptive and neither the intensity of pain, the temperature or the SR and leucocyte count are suitable for differential diagnostic purposes. For instance torsion of the testis to occur during the neonatal period need not at all be accompanied by pain (6). The age of patients may provide certain clues. Fig. 1 illustrates the age distribution characteristic of epididymitis and torsion of the testis. While epididymitis mainly is seen in patients in the postpubertal age group torsion of the testis will generally be encountered in children aged 10 to 15 years although it may also occur during the neonatal period even during intrauterine life upon which the lesion will be manifest in the child at birth (1). As torsion of the

Table 1 *Material*

Case no	Age	Diagnosis	Microscopic findings
1	6 years	Epididymitis Allergic purpura (Schönlein-Henoch)	Marked eosinophilia Marked vascular necrosis Normal epithelium in ductuli
2	3½ years	Epididymitis Allergic purpura (Schönlein-Henoch)	Light eosinophilia Light vascular necrosis Normal epithelium in ductuli
3	3½ months	Idiopathic epididymitis Chronic digestive disorder (milk allergy?)	Unspecific inflammation Necrosis of epithelium in ductuli
4	2½ months	Pyogenic epididymitis (coli)	Biopsy specimen too small
5	5 weeks	Idiopathic epididymitis	Unspecific inflammation Necrosis of epithelium in ductuli
6	5 years	Postoperative epididymitis	

testis often may result in atrophy of the testes emergency operation is generally indicated with a view to escaping this (1 3)

Torsion of appendix testis gives similar al though more moderate symptoms Tumours will not initially give either pain or oedema Hydroceles are pellucid they involve no pain and the scrotal content is not increased Allergic or idiopathic oedema of the scrotum will often produce considerable oedema the scrotal content will remain normal and the condition may often develop as a complication in infections of the upper respiratory tracts In cases of haematocoeles and incarcerated inguinal hernia the anamnesis may serve as guidance

Orchitis may occasionally be accompanied by epididymitis (1) Such orchitis may be excited by parotitis virus coxsackie virus varicella or mononucleosis but it is rarely seen in children One case of epididymitis secondary to parotitis but absence of orchitis is on record (5) Another although rare causative factor is gonorrhoea which was demonstrable in 2 of adult patients with epididymitis (12) The aetiology of epididymitis remains obscure in more than 50% of all cases however (8 12) An epidemic of such idiopathic epididymitis is on record (14)

The aetiology of the disease is a much debated question (4 8 11 12 14 15 16) Campbell (4) emphasizes that infection via the

urinary tract may be a significant aetiological factor Mittenmeyer et al (12) observed that 20% of their patients with epididymitis had a concurrent infection of the urinary tract but only 2 of the patients were found to have malformations of the urinary tract Eegholm (8) found similar conditions he declared that haemorrhagic cystitis also might be a common finding Similar features may apply to children (1 6) A report appeared in 1969 according to which epididymitis had been diagnosed in three children in the prepubertal age group (2) vesico-ureteral reflux and other malformations of the urinary tract were demonstrable in all three children

It is still an object of much discussion how an infection may spread to epididymis but there is every probability that it may proceed via lymphogenic or more rarely haematogenic routes (15 16) Reflux of infected urine through the deferent duct occurs exclusively if the latter is dilated and without tonus for instance as a sequela of protracted infection (16) An intensified intravesical pressure will under normal conditions result in an intensified tonus in the neck of the bladder and occlusion of the ejaculatory duct just as at the ureteropelvic junction (11) The ciliary function in the ejaculatory duct serves also to prevent reflux of urine Ligature of the deferent duct in patients with recurrent epididymitis does not lower the in

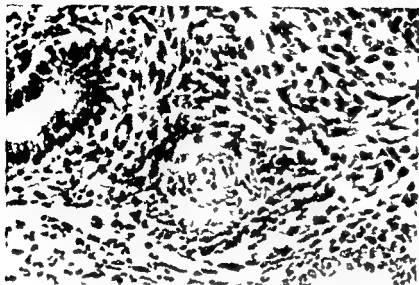


Fig. 2 Marked vascular necrosis and normal epithelium in ductuli epididymis (Case 1)

cidence of recurrences and epididymitis is a common occurrence in vasectomized patients (16).

Infection of the urinary tract did not in any case accompany the epididymitis in the patients discussed here, such infection was not either manifest prior to admission or during the interval between hospitalization and follow up. In travenous urography showed normal conditions in five of the patients, one patient was found to have double kidneyanlage on one side but no other anomalies. This patient was the only one in whom cysto urethrography of micturition was done, but reflux was not observed. In four of the children the SR or the leucocyte count remained normal while the SR was moderately elevated in two. It is remarkable that three of the children, including the two with

Schonlein Henoch's purpura presented increased thrombocyte counts over longer periods. Patients suffering from the latter disease have usually normal thrombocyte values. According to a recent publication thrombocytosis seems to be a common occurrence in patients with rheumatoid arthritis (10). The significance of this postulation remains to be defined but the phenomenon may be linked up with an immunological process.

The patients were examined after intervals covering from 6 months up to 3 years after hospitalization the scrotum had normalized in all cases and there had not been any recurrences.

The microscopic picture of epididymis in the two children with Schonlein Henoch's purpura was distinctly different from that of the other



Fig. 3 Unspecific inflammation with necrosis of epithelium in ductuli epididymis (Case 5)

patients (Table 1) The vascular wall was the site of necrosis there was marked oedema of the wall and the endothelial cells were partly destroyed The vascular wall and the stroma were sites of inflammatory infiltrations composed of polymorphonuclear leucocytes with numerous eosinophil cells as well as of lymphocytes and plasma cells The columnar epithelium in the ductules was well preserved The changes were similar to those known from Schönlein-Henoch's purpura they were most pronounced in case 1 (Fig 2) less pronounced in case 2 The other two biopsies revealed non specific inflammatory changes but well preserved vessels; damaged epithelium in the ductules and inflammatory infiltration in the stroma with few eosinophil cells These changes were most pronounced in case 5 (Fig 3) less pronounced in case 3

Whenever a boy is found to have a painful and swollen scrotum torsion of the testis or incarcerated hernia should first be suspected and as a rule surgical intervention will be indicated Microscopy and culture of urine should be performed and if the diagnosis of epididymitis is verified the urinary tracts should be examined with a view to malformations If the patient is afflicted with a concurrent Schönlein-Henoch's purpura there is every probability that it is a matter of an epididymitis involved as a complication in this disease

SUMMARY

The case histories of six children with epididymitis are submitted In 2 cases the epididymitis formed part of Schönlein-Henoch's purpura and microscopy of biopsies from the epididymis showed changes characteristic of the latter disease In 1 case the epididymitis was of suppurative nature and in 1 case it represented a complication following orchiopepy In the remaining 2 cases the aetiology of the disease remained obscure

Infection of the urinary tract was not observed in any of the cases and the urinary tract was normal in all except one in whom double anal are of one of the kidneys was demonstrable

Three of the patients including the two with Schönlein-Henoch's purpura had thrombocytosis The aetiology and the differential diagnoses are briefly discussed It is emphasized that the suspicion primarily should be focussed on torsion of the testis or incarcerated hernia whenever a boy is found to have a tender and swollen scrotum It is emphasized also that the two latter diagnoses as a rule can be excluded by operation

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CASE REPORT

A CASE OF PRADER-WILLI SYNDROME IN A GIRL WITH A SMALL EXTRA CHROMOSOME

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In 1956 Prader Labhart and Willi described 9 cases of a syndrome comprising mental deficiency short stature obesity hypogonadism and muscular hypotonia. A tendency to diabetes (9) and acromicria (6) were subsequently added to the list of characteristic clinical features. Since these reports a variety of other findings have been described in published cases which have been extensively reviewed and discussed (2, 4, 7, 11). We are aware of 109 recorded examples of the syndrome of which 57 had been karyotyped. Six of these are reported to have distinct chromosomal abnormalities of different kinds: one with an extra Y chromosome (5), another with an additional G chromosome (12), a mosaic with D/E translocation (12), a D/D translocation (1), a mosaic D trisomy (10) and a mosaic G/G translocation (2). Further instances of chromosomal aberrations in persons with the Prader-Willi syndrome may help to clarify the obscure relationship of such aberrations to the syndrome. We therefore report an example of the syndrome in a patient with a small extra chromosome.

CASE REPORT

J O (II), (a female born 1949) is the youngest of five surviving children (see Pedigree in Fig 1). The mother's first pregnancy (II) terminated in a stillbirth at 36 weeks and the third and fourth resulted in twin births with one twin in each pair stillborn and malformed (II and II). Unfortunately no information is available on the type of malformations in these two individuals. J O's surviving sibs are all apparently normal though her sisters (II and II) did not menstruate until 15-16 years of age. Her mother's menarche occurred at the age of 17 years followed by infrequent periods and marked gain in weight for the next 2 years. The father of the proposita died at 65 years of pneumonia; he had one son by a previous marriage. The ages of the mother and father at the birth of the proposita were 44 and 63 years respectively. There is no history of consanguinity in the family.

J O was born at term by breech delivery after a rapid labour. The pregnancy was uneventful apart from hypertension. The infant weighed 2800 g, was hypotonic, did not cry and was unable to suck. Feeding difficulties persisted neonatally and the child lost weight. Her condition appeared to improve thereafter. However when she started school at 5 years she was found to be mentally retarded and was transferred to a training centre. Increasing obesity was noted at 8 years and she developed a voracious appetite. The development of secondary sexual characteristics was delayed.

J O was referred to us at the age of 17 years (see Fig 2) for investigation of her amenorrhoea and obesity. She was found to be mentally retarded with an estimated IQ of 40 and of a pleasant disposition. She was short (height 140 cm) with marked generalized obesity (weight 72.3 kg) particularly affecting the cheeks, chin, lower trunk and legs. The head was small and rounded with the following measurements: circumference 520 mm, length 172 mm, breadth 142

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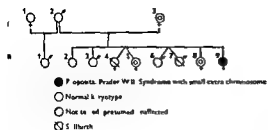


Fig 1 Pedigree of family

mm height, 120 mm cephalic index 0.83. She was myopic and had a small mouth, high palate and grossly hypoplastic and carious teeth. She was hypotonic, the legs showed genu valgum and she walked with a lumbering gait. Other features noted were acromicria, hypogonadism, abdominal striae, retracted nipples and scanty axillary and pubic hair. She had not yet menstruated.

Chromosomal findings

Karyotypes prepared from cultures of peripheral lymphocytes and skin fibroblasts showed a diploid number of 47 chromosomes in all cells examined. The extra chromosome was consistently found to be slightly smaller and more metacentric than the chromosomes of the G group (see Fig 3). In some cells the additional chromosome showed satellites and satellite association. Apart from occasionally dimorphic number 16 chromosomes, no other peculiarities were detected. Karyotypes from lymphocyte cultures taken from other available members of the family appeared to be normal. The findings are summarised in Table 1.

Other investigations

The urinary follicle stimulating hormone level was 6 mouse units/24 hours. A glucose tolerance curve showed mild intolerance to glucose. Radiographs of the hands and wrists indicated a normal bone age. Dermatoglyphic examination revealed no notable differences between the proposita and other members of her family.

DISCUSSION

The main findings in the present case are compared, in Table 2, with those commonly re-

ported in previously published cases of Prader Willi syndrome. Clinically she appears to be a typical example of the syndrome despite the presence of the extra chromosome.

Cases of the syndrome are more likely to be recorded if an associated chromosomal aberration is present. Nevertheless, the occurrence of 7 reported instances, including the present one of chromosomal abnormality among 58 karyotyped patients with the syndrome, is impres-

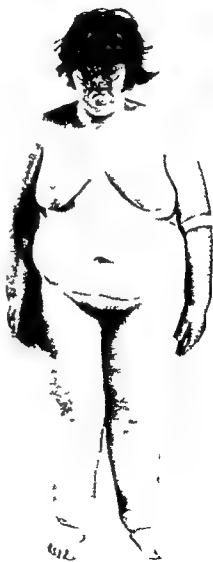


Fig 2 Propositia at 17 years of age

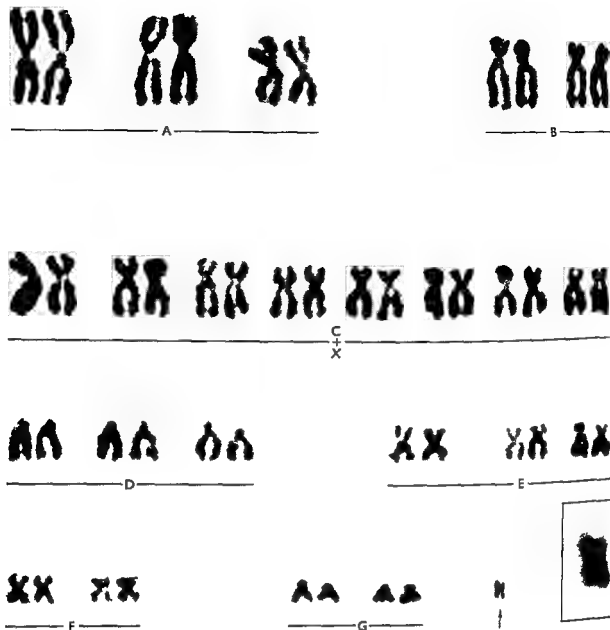


Fig 3 Karyotype of proposita from a leucocyte culture showing 47 chromosomes. The extra chromosome is arrowed with an enlargement adjacent

sive. By contrast only 10 of 1788 live born babies studied by Court Brown & Smith (3) showed definitely abnormal karyotypes. The identity of the extra chromosome in our patient is uncertain. If it is a fragment as seems likely the presence of satellites implies an origin from one of the D or G group of chromosomes. The other examples of the syndrome reported to

show chromosome abnormalities involve the D or G group in 5 cases (1, 2, 10, 12) and a Y chromosome in one (5). Although there is no consistency in these findings it may be important that acrocentric chromosomes are most commonly involved.

Though a genetical basis for the Prader-Willi syndrome has received consideration (4)

Table 1 Chromosomal findings in *proposita* and relatives

Case	Pedigree no (see Fig 1)	Culture	Cells counted	Karyotype	Comments
Proposita	II	F	30	47/XX	Extra small satellited metacentric
Proposita	II	L	30	47/XX	Extra small satellited metacentric Occasional dimorphic no 16s
Mother	I	L	30	46/XX	Normal female karyotype Occasional dimorphic no 16s
Brother	II ₁	L	40	46/XY	Normal male karyotype Occasional dimorphic no 16s
Sister	II	L	30	46/XX	Normal female karyotype Occasional dimorphic no 16s
Sister	II	L	30	46/XX	Normal female karyotype Occasional dimorphic no 16s

L = Leucocyte culture F = Fibroblast culture

Table 2 Main clinical findings in *proposita* compared with those commonly recorded in published cases of Prader Willi syndrome

Reported findings	Findings in <i>proposita</i>
Mental retardation	+
Short stature	+
Obesity (especially affecting lower trunk legs cheeks and chin)	+
Hypogonadism	+
Hypotonia	+
Acromicria	+
Abnormal glucose tolerance	+
Birth weight below 3 000 g	2 773 g
Neonatal feeding difficulties	+
Excessive appetite in childhood	+
Small head	+
Ocular anomalies	- (myopia)
Hypoplastic carious teeth	+
High arched palate	+
Micrognathia	-
Genu valgum	-
Delayed development of secondary sexual characteristics	-
Urinary gonadotrophins increased or decreased	Decreased
Retarded bone age	- (X ray hand and wrist)

Most characteristic features

the aetiology of the syndrome is as yet obscure. Clarification of the possible significance of the findings mentioned above must await the accumulation of further data.

SUMMARY

A characteristic case of Prader Willi syndrome is reported in a girl who showed additionally

the presence of a small extra chromosome possibly a fragment of a D or G group chromosome. Available members of the *proposita*'s family were found to be phenotypically and cytologically normal. The case is compared with those previously reported.

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CASE REPORT

GIANT HAEMANGIOMA WITH A DISORDER OF COAGULATION

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Haemangioma is rarely complicated by disorders of blood coagulation. Such a complication was first described by Kasabach & Merritt (21) in 1940 since when about 80 cases have been reported (20, 35-38). About 80% of these cases have been seen in children below 1 year.

It was not until some 10 years ago that coagulation disorders attending haemangioma began to receive serious attention. Of 19 cases (3-5, 7-9, 20, 35, 39, 41, 42, 45) studied extensively thrombocytopenia ($< 100\,000$) occurred in 17, a decrease in factor V ($< 80\%$) in 10 and a decrease of factor VIII ($< 60\%$) in 5. A low fibrinogen concentration (< 260 mg/100 ml) was found in 17 cases and increased fibrinolysis in 7. Platelet and fibrinogen turnover studies have been performed in a few cases. Thus Blix & Aas (7) found a rapid disappearance of ^{51}Cr labelled platelets from the blood and an uptake of the activity by the tumour. Using ^{125}I labelled fibrinogen Wochner (43) demonstrated a high disappearance rate of fibrinogen which was normalised by administration of heparin. Hillman (19) reported an increased uptake of labelled fibrinogen over the haemangioma even when allowance was

made for the increased local blood volume due to the tumour.

The rapid disappearance of the labelled platelets and the labelled fibrinogen in these cases and the low platelets, factor V and fibrinogen values reported in several other cases have often been interpreted as manifestations of intravascular coagulation in the haemangioma with consumption coagulopathy as a result. The increased fibrinolytic activity recorded in some cases has also been regarded as secondary to the intravascular coagulation process. Such mechanisms as mechanical destruction of the platelets in the tumour and destruction of the fibrinogen due to fibrinolytic activators produced by the tumour i.e. mechanisms other than intravascular coagulation have so far not been considered in the causation of the coagulation disorders complicating haemangioma.

This paper reports on a case of haemangioma with a disorder of the coagulation studied repeatedly during 2 years with various methods including the estimation of the turnover of platelets and fibrinogen with the aid of radioisotopes.

CASE REPORT

The patient was a girl born in September 1967. The parents appeared healthy and pregnancy and delivery

This investigation was supported by grants from the Swedish Medical Research Council (B70-19X 87-06C).



Fig. 1 Haemangiomatous arm at March 1970

had been uncomplicated. Nothing remarkable was noticed until the child was 3 months when a small blue patch was observed on the left elbow. This discoloration grew rapidly in size and within 3 weeks the entire arm was involved by a blue-red tumour extending down to the wrist. Small petechiae had by then appeared on the neck and chest. On first admission to hospital (Lund, January 1968) the platelet count ($20\,000$ platelets/mm³) and the haemoglobin concentration (8 g/100 ml) were low. A biopsy speci-

men showed a picture resembling that of Kaposi's sarcoma but without convincing signs of malignant cells. The histological diagnosis was haemangioma. Angiography showed a soft tissue tumour with early shunting to venous cavities. Subsequent examination of the bone marrow showed an increased number of megakaryocytes. Roentgen irradiation of the haemangioma in January 1968 with a total dose of 805 R produced no demonstrable effect.

By the time of admission to Malmö General Hos-

Table 1 Coagulation studies

	May 1968	April 1969		May ^a 1969	November ^a 1969	March ^a 1970	Normal range
		Healthy arm	Angiom arm				
Platelet number per mm ³	22 000	28 000	20 000	74 000	20 000	30 000	150 000-430 000
Bleeding time							
Duke min	13	11			10	10	1-4
Ivy min	> 30						6-15
Coagulation time							
glass min	9						8-14
plastic min	26						15-25
One stage prothrombin time sec	15	16	12	14	16		14-16
Recalcification time sec	157	125	135	152	140	180	170-180
P & P	90	114	114	120	105	79	80-110
Factor I	100	75	80	82	106	104	80-110
Factor VIII	113	197			160	82	60-140
Fibrinogen g/100 ml	0.31	0.20	0.14	0.24	0.24	0.30	0.16-0.34
Plasminogen							
clot method	88	120	103	60	104		60-140
immunochem method	88		90				
α_2 macroglobulin	227	220			233		
Fibrinolytic activity on unheated plates							
plasma mm ²	30	35	0	18		0	0-50
resusp. euglob. prec mm ³	40	125	88	66		10	0-70
Fibrinolytic split products mg/100 ml	3	3.9	6.4	1	1	Traces	0

^a During treatment with EACA

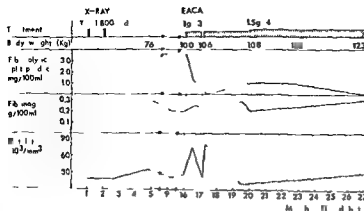


Fig 2 The course of the disease

pital in May 1968 the haemangioma had involved half of the upper arm and two thirds of the lower arm. The arm was thickest in the region of the elbow. The skin was somewhat uneven, relatively warm, purplish red and hairs were growing sparsely from its surface. The arm felt abnormally firm to hard. The arm was flexed 60° and stiff. About 2 cm above the elbow joint the affected arm measured 24 cm in circumference compared with 14.5 cm on the other side. Haemoglobin 10.6 g/100 ml, Haptoglobin 186 mg/100 ml. Other routine laboratory studies revealed nothing remarkable. During the rest of the patient's stay in hospital the haemoglobin level was about 1 g/100 ml. The coagulation of the blood was studied repeatedly (Table 1).

Owing to its increased vascularity the affected arm grew quicker than the contralateral arm. Thus 9 months after onset the humerus was 9 mm, the ulna 8 mm and the radius 7 mm longer on the affected side.

Since the patient showed high fibrinolytic activity in the tumour tissue and split products in the blood treatment was started with EACA (Epsikapron®) in a dose of 1.5 g 3 in April 1969. During treatment the haemangioma decreased peripherally and the discoloration of the skin became less intense. At the same time the platelet count increased (Table 1, Fig. 2). After the patient had received EACA at home for 1 month the preparation was unintentionally withdrawn. Three days later bleeding occurred in the haemangioma and the patient was re-admitted to hospital. On admission it was found that the platelet count had again fallen. Resumption of treatment with EACA rapidly controlled the bleeding, the platelet count increased though only temporarily (Fig. 2). During continued treatment with EACA the coagulation of the blood was examined on 3 occasions viz 19, 21 and 27 months after the onset of the disease (Fig. 2, Table 1). The size of the haemangioma successively decreased more in area than in thickness.

The circumference of the arm 4 cm above the elbow joint was thus still 24 cm but the tumour now involved only one third of the lower arm and barely one third of the upper arm. The corresponding circumference on the right side had now increased to 15.5

cm. The skin was somewhat purplish red but did not feel abnormally warm. The arm was still as firm as before. Some areas of normal skin were seen on the flexor side of the tumour (Fig. 1).

The roentgenological difference in length of the humerus had now increased to 15 mm while that of the ulna and radius had decreased to 5 mm and 3 mm respectively.

The physical development of the child was otherwise normal.

LABORATORY STUDIES

Methods

Platelet count was made by the method of Björkman (6).

Bleeding time (Duke's technique) was determined bilaterally. Standardized haemolets (Sera Sharp blood lancets, Proper MRG Co, New York) were used. The bleeding time was also determined by the method of Ivy as modified by Nilsson et al. (31).

Prothrombin factor VII and factor X were measured by the P & P method of Owren & Aas (32). Factor V principally according to Wolf (44) and factor VIII according to Nilsson et al. (28).

Fibrinogen was determined in the way described by Nilsson & Ölow (29). Only fibrinogen values for blood collected with EACA are given.

Plasminogen was measured both by a clot method (30) and by an immunochemical method (16).

The α -macroglobulin concentration was determined by the method of Ganrot (5).

Fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated bovine fibrin plates as described by Nilsson & Ölow (29).

Fibrinolytic split products were determined with the immunochemical method of Nilsson (27). The analysis was performed on serum samples obtained from blood collected in tubes containing EACA.

The plasminogen activator content of the venous wall was measured as the amount in a roughly 0.5 cm long biopsy specimen obtained from the haemangioma with the patient under general anaesthesia. The fi



Fig. 1 Haemangiomatic arm at March 1970

had been uncomplicated. Nothing remarkable was noticed until the child was 3 months when a small blue patch was observed on the left elbow. This discoloration grew rapidly in size and within 3 weeks the entire arm was involved by a blue-red tumour extending down to the wrist. Small petechiae had by then appeared on the neck and chest. On first admission to hospital (Lund, January 1968) the platelet count (20 000 platelets/mm³) and the haemoglobin concentration (8 g/100 ml) were low. A biopsy speci-

men showed a picture resembling that of Kaposi's sarcoma but without convincing signs of malignant cells. The histological diagnosis was haemangiomaticus. Angiography showed a soft tissue tumour with early shunting to venous cavities. Subsequent examination of the bone marrow showed an increased number of megakaryocytes. Roentgen irradiation of the haemangioma in January 1968 with a total dose of 800 R produced no demonstrable effect.

By the time of admission to Malmö General Hos-

Table 1 Coagulation studies

		April 1969		May ^a 1969	November ^a 1969	March ^a 1970	Normal range
	May 1968	Healthy arm	Angiom. arm				
Platelet number per mm ³	22 000	28 000	20 000	74 000	20 000	30 000	150 000-400 000
Bleeding time							
Duke min	13	11			10	10	1-4
Ivy min	>30						6-15
Coagulation time							
glass min	9						8-14
plastic min	26						15-25
One stage prothrombin time sec	15	16	12	14	16		14-16
Recalcification time sec	157	125	135	152	140	180	170-180
P & P	90	114	114	120	105	79	80-110
Factor V	100	75	80	82	106	104	80-110
Factor VIII	113	197			160	82	60-140
Fibrinogen g/100 ml	0.31	0.20	0.14	0.24	0.24	0.30	0.16-0.34
Plasminogen							
clot method	88	120	103	60	104		60-140
immunochem. method	88		90				
α_2 macroglobulin	227	220			233		
Fibrinolytic activity on unheated plates							
plasma mm ²	30	35	0	18		0	0-50
resusp. euglob. prec. mm ²	40	125	88	66		10	0-70
Fibrinolytic split products mg/100 ml	3	3.9	6.4	1	1	Traces	0

^a During treatment with EACA

gioma. The haemangiomatous arm was about twice as thick as the other arm and therefore contained a much larger amount of blood. The values found for radioactivity on either side are therefore not strictly comparable. There was however a continuous increase in activity over the haemangiomatous arm during the first 2-3 hours when the activity in the blood was decreasing. This indicates that the higher activity over the haemangiomatous arm was due not only to the larger size of the arm but to a true uptake.

The rapid disappearance of the platelets in the haemangioma may be explained either by consumption of the platelets in the tumour due to formation of microthrombi and/or by

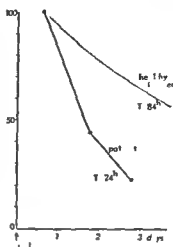


Fig 5 Disappearance rate of labelled fibrinogen in the patient and in healthy adults

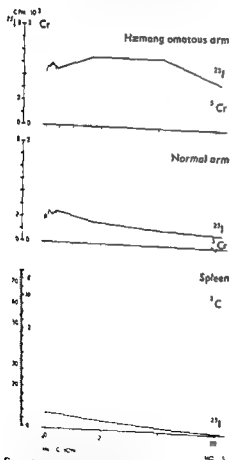


Fig 4 ⁵¹Cr activity over haemangiomatous arm, normal arm and over the spleen after injection of labelled fibrinogen

mechanical breakdown of the platelets in the dilated sinusoids in the haemangioma. The finding of normal values for the various coagulation factors in association with a very low platelet count argues against a consumption syndrome.

The parallel course of the uptake of Cr activity by the haemangioma and the spleen is however striking and suggests that the haemangioma behaves like the spleen. It thus appears probable that some of the platelets are destroyed mechanically in the dilated sinusoids in the haemangioma. On the other hand there was definitely some evidence of intravascular coagulation. A moderate consumption of coagulation factors need not cause a coagulation defect for as known from turnover studies of fibrinogen (23) the body can compensate for an increased loss by an increased synthesis. In

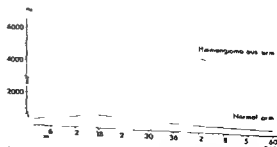


Fig 6 ¹²⁵I activity over haemangiomatous arm and normal arm after injection of labelled fibrinogen

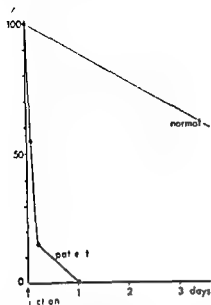


Fig 3 Disappearance rate of labelled platelets in the patient and in healthy adults

fibrinolytic activity was demonstrated with Pandolfi's modification of the histochemical method of Todd (40) and assessed according to the grading used by Pandolfi *et al* (33). In normal individuals the plasminogen activator content of superficial arm veins is 6–11 arbitrary units.

Platelet turnover was estimated with the aid of ^{51}Cr labelled (1) platelets from the patient's mother. Fibrinogen was labelled with ^{125}I essentially according to the capillary jet technique of McFarlane (25) as modified by Blomback *et al* (11) but with the use of iodine monochloride (ICl) as the iodine source instead of molecular iodine (I_2) (26). The fibrinogen was prepared from plasma (10) from a few selected donors. The radioactivity was determined (23) in a fibrin solution.

External counting after injection of ^{51}Cr labelled platelet and ^{125}I labelled fibrinogen respectively was performed with a collimator. The activity was measured over the haemangiomatous arm over the normal arm and in the platelet study also over the spleen.

RESULTS

The results of the coagulation studies are given in Table 1 and in Fig 2. On admission to hospital in May 1968 the patient had thrombocytopenia and prolonged bleeding time (Duke's and Ivy's technique). The fibrinogen, prothrombin, factor V and factor VIII and plasminogen values were normal. No increased fibrinolytic activity could be demonstrated in the circulating blood, which however contained fibrinolytic split products. The plasmin

ogen activator content of the walls of the tumour vessels in the biopsy specimen of the tumour was high (7 arbitrary units). On one occasion (April 1969) when samples were obtained from both arms the sample from the affected side contained less fibrinogen and more fibrinolytic split products than that from the contralateral side.

The results of the platelet turnover studies are given in Fig 3. The activity fell to about 50% within 2 hours compared with 110 hours in normal adults. Most of this activity was taken up by the affected arm (Fig 4). This uptake started early after the injection and parallel to the uptake in the spleen.

Fig 5 gives the results of turnover studies with ^{125}I labelled fibrinogen which indicated a half time of about 24 hours compared with 84 hours in three healthy adults studied in this laboratory. The uptake by the haemangiomatous arm was high and progressed during the first 18 hours after the injection (Fig 6).

Administration of EACA was followed by an increase of the platelet count and a decrease of the amount of split products in the blood (Fig 2). The fibrinogen level and the other coagulation factors remained largely unchanged. After 1 month EACA was withdrawn and the platelet count fell but rose after resumption of treatment with the drug. The platelet count afterwards gradually fell despite treatment with EACA (Fig 2). Only traces of split products were demonstrable in the last samples. As pointed out above the haemangiomatous arm continuously decreased in size during EACA therapy (Fig 1).

DISCUSSION

The coagulation studies in this patient with a giant haemangioma of the left arm revealed severe reduction in the number of circulating platelets. Judging from the turnover studies this thrombocytopenia was obviously due to a rapid disappearance of platelets. The external counting indicated that the labelled platelets were to a large extent taken up by the haeman-

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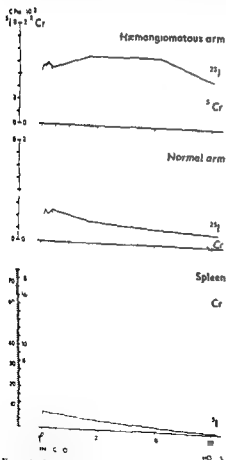


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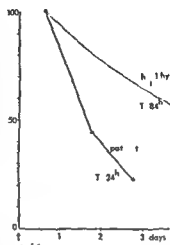


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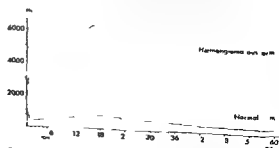


Fig 6 ^{125}I activity over haemangiomatous arm and normal arm after injection of labelled fibrinogen.

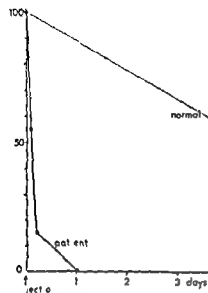


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while intravascular coagulation played only a subordinate role. On the other hand the fibrinolytic activity in the tumour vessels was found to be high which together with the occurrence of fibrinolytic split products in the blood was considered to indicate treatment with inhibitors of fibrinolysis (EACA). During this treatment the angioma decreased in size. It would therefore appear advisable to investigate the mechanisms of the coagulation disorders in these patients before deciding upon treatment.

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this patient the turnover rate of fibrinogen was relatively high but not as high as that of platelets. Though no data are available concerning the turnover rate of these components in healthy children there is no reason to believe that they are significantly shorter than in adults. The external counting indicated an uptake of fibrinogen by the haemangioma. This uptake occurred later than that by platelets and seemed to continue for a longer time indicating the presence of a coagulation process in the haemangioma.

The presence of fibrinolytic split products in the peripheral blood showed the occurrence of degradation of fibrinogen and/or fibrin. The biopsy of the tumour showed high activity of activators of fibrinolysis in the walls of the small vessels in the angiomatous tissue. Blood from the affected arm contained more split products and less fibrinogen than did blood from the other arm. Taken together these findings indicated fibrinolysis in the haemangioma. Activators must be released from the vessels and after activation of the fibrinolytic system precipitated fibrin and possibly also fibrinogen are degraded.

The decrease in the amount of split products following treatment with EACA provides further evidence of involvement of a fibrinolytic component. The use of heparin has been recommended in the treatment of the coagulation disturbances in patients with this type of haemangioma. It has been claimed that heparin inhibits consumption of the coagulation factors (7, 12, 19). But no effect on the structures of the haemangioma have been observed. It might be mentioned that in a recent case of haemangioma with consumption of coagulation factors treatment including heparin therapy could not ward off a fatal issue (20).

We refrained from using heparin in our case because coagulation and turnover studies had indicated that the main cause of the thrombocytopenia in our patient was mechanical destruction of the platelets in the haemangioma and that intravascular coagulation played only a minor role and second because administra-

tion of heparin to patients with a low platelet count involves a high risk of bleeding. Third, the high fibrinolytic activity in the tumour vessels and the occurrence of fibrinolytic split products in the blood suggested rather the use of a fibrinolytic inhibitor in the treatment of this case. We therefore decided to use EACA in order to inhibit the fibrinolytic activity in the affected arm and thereby possibly facilitate thrombotisation of the haemangioma. Since this form of tumour often regresses spontaneously it is not possible to say anything definite about the effect of EACA except that it had no side effects. On the contrary the tumour decreased in extent and the discoloration of the skin became less intense. The girl had no serious bleeding episodes except during unintentional withdrawal of EACA.

In cases of haemangioma with coagulation disorders we think it is worth while to try to find out to what extent the disorder is due to intravascular coagulation, mechanical destruction of the platelets in the tumour and destruction of fibrinogen or fibrin by fibrinolytic activators produced by the tumour and to treat the patients accordingly.

SUMMARY

The mechanism of the coagulation disorder in an infant with a large haemangioma of the right arm has been studied. The disorder was characterised by thrombocytopenia, prolonged bleeding time and the occurrence of fibrinolytic split products in the blood. The levels of various coagulation factors were normal. The mechanism of the disorder was further investigated by injection of ^{51}Cr labelled platelets which disappeared very rapidly from the circulation owing to the uptake by the haemangioma. Injection of ^{125}I labelled fibrinogen revealed a considerable increase in disappearance rate which however was not as high as for ^{51}Cr labelled platelets. Treatment with heparin in this case appeared inadvisable because the thrombocytopenia was found to be due mainly to mechanical destruction in the haemangioma.

while intravascular coagulation played only a subordinate role. On the other hand the fibrinolytic activity in the tumour vessels was found to be high which together with the occurrence of fibrinolytic split products in the blood was considered to indicate treatment with inhibitors of fibrinolysis (EACA). During this treatment the angioma decreased in size. It would therefore appear advisable to investigate the mechanisms of the coagulation disorders in these patients before deciding upon treatment.

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CASE REPORT

OCULODENTODIGITAL DYSPLASIA SYNDROME

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Plastic and Reconstructive Surgery Hacettepe University
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Since the original description of this clinical entity by Lohmann in 1920 (7) additional cases from various parts of the world have been published (1 2 8 9 10) Oculodentodigital dysplasia syndrome (ODD) is characterized by a thin nose with hypoplastic alae microphthalmus microcornea and other ocular anomalies syndactyly and camptodactyly of the fourth and fifth fingers clinodactyly of the fifth finger and hypoplasia of the enamel (5) So far there have been 44 cases in 21 families described in literature including ours (11) The purpose of this report is to present a case where characteristic features of the ODD syndrome are present

CASE REPORT

The proband is a 13 months old boy was a full term product of a normal pregnancy and delivery The mother was 24 and the father was 39 years old It was the first pregnancy of the mother she had two others subsequently one of which resulted in stillbirth and the other in an abortion The patient's growth and development were within normal limits The parents were normal except that the father had a thin nose with moderate hypoplastic alae There was no parental consanguinity and the family history for eye and finger anomalies was negative

Clinical findings Physical examination revealed a well-developed boy with multiple congenital anomalies His height was 90 cm (75 percentile) weight 13 kg (50 percentile) and head circumference 49 cm (50 percentile) The head was elongated vertically the hair was dry and brittle He displayed bilateral

epicanthal folds hypotelorism small aperture of the eyelids bilateral microphthalmia microcornea and strabismus The nose was thin with marked hypoplasia of the alae and narrow nostrils (Fig 1) Bilateral syndactyly and camptodactyly of the fourth and fifth fingers and bilateral clinodactyly of the fifth fingers were noted (Fig 2) Dermatoglyphic studies of the finger tips palms and soles revealed no abnormalities Dental examination revealed two fractured teeth and very poor oral hygiene There was nothing to suggest that there was enamel hypoplasia

His motor and mental development were within normal limits for his age (Gesell developmental test) His responses during sound field hearing testing were around 20 decibel level This finding and the parental history were interpreted as an indirect evidence that his hearing was normal

Röntgenographic examination of the feet showed only two phalanges in the fifth toes bilaterally Metatarsal and metatarsal bones were broad and short In addition the middle phalanges of the fourth and fifth fingers were hypoplastic and there was medial angulation of the proximal interphalangeal joints of the fourth fingers bilaterally (Fig 3)

Chromosome analysis from cultured peripheral blood leukocytes was normal

DISCUSSION

The clinical and the roentgenographic findings in our case are typical of the ODD syndrome The findings can be summarized in the following categories

Head Typical thin nose with hypoplastic alae and narrow nostrils epicanthal folds and hypotelorism constitute the characteristic facial appearance Microcornea is known to be a frequent sign (11) Microphthalmus is also fre

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CASE REPORT

OCULODENTODIGITAL DYSPLASIA SYNDROME

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Since the original description of this clinical entity by Lohmann in 1920 (7) additional cases from various parts of the world have been published (1 2 8 9 10) Oculodentodigital dysplasia syndrome (ODD) is characterized by a thin nose with hypoplastic alae microphthalmus microcornea and other ocular anomalies syndactyly and camptodactyly of the fourth and fifth fingers clinodactyly of the fifth finger and hypoplasia of the enamel (5) So far there have been 44 cases in 21 families described in literature including ours (11) The purpose of this report is to present a case where characteristic features of the ODD syndrome are present

CASE REPORT

The propositus a 73 months old boy was a full term product of a normal pregnancy and delivery The mother was 24 and the father was 39 years old It was the first pregnancy of the mother she had two others subsequently one of which resulted in still birth and the other in an abortion The patient's growth and development were within normal limits

The parents were normal except that the father had a thin nose with moderate hypoplastic alae There was no parental consanguinity and the family history for eye and finger anomalies was negative

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His motor and mental development were within normal limits for his age (Gesell developmental test) His responses during sound field hearing testing were around 70 decibel level This finding and the parental history were interpreted as an indirect evidence that his hearing was normal

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Fig 1 Facial appearance of the patient

quently seen and in some cases it is associated with small orbits (6). The lid aperture, interpupillary and intercanthal distances are reduced (5). Congenital cataract, colobomas of

the various ocular structures, strabismus and remnants of the pupillary membrane are frequently present (5, 8). In addition, in our case a corneal opacity was noted, which has not been described before.

Hair is brittle, sparse and dry (5). Head circumference is usually normal. Microcephaly was noted only in 2 cases in literature (6, 12). In most cases, including ours, motor and mental development were within normal limits.

Dental findings. Teeth show generalized enamel hypoplasia in most cases (2, 5, 8, 10, 12). However, in a very few cases the teeth were noted to be normal. In our case, normal primary dentition does not necessarily rule out the future occurrence of enamel hypoplasia of the primary or the permanent teeth.

Extremities. Bilateral syndactyly and camptodactyly of the fourth and fifth fingers and clinodactyly of the fifth fingers are found in almost all cases.

Genetics. We found twenty-one families with the ODD syndrome in the studied literature. In six, the inheritance pattern appeared to be autosomal dominant. The rest were sporadic (11). X-linked inheritance can be ruled out by the presence of an almost equal number of affected males and females and the presence of father-to-son transmission of the trait. Besides the cases which showed autosomal dominant

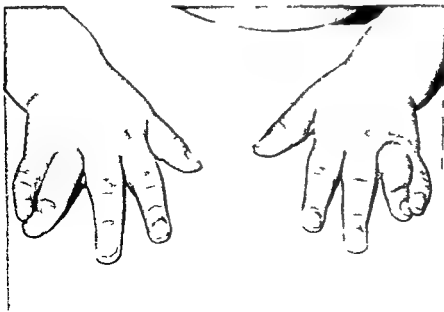




Fig 3 Roentgenogram of the hands showing hypoplasia of the middle phalanges of the 4th and 5th fingers bilaterally

inheritance there have been sporadic cases showing parental consanguinity. The cases might indeed be sporadic or they might be considered to show autosomal recessive inheritance (3, 4). However, the generally accepted pattern of inheritance is an autosomal dominant (11).

The presence of sporadic cases as well as familial ones with probably different inheritance patterns can be explained in one of the following ways:

1 This syndrome consists of several clinically similar but basically different entities which show genetic heterogeneity.

2 It shows autosomal dominant inheritance with incomplete penetrance and/or varying expressivity in some generations. For example, in our case the father's thin nose and hypoplastic alae are suggestive of incomplete expressivity of the trait in the family. The remainder of the sporadic case is the result of a new mutation which may eventually be proven to have dominant inheritance pattern.

3 The sporadic cases are due to unknown mutagenic agents and are phenocopies.

Analyses of the cultured peripheral blood leukocytes have been done in some of the cases in the literature, none of which revealed any visible chromosomal abnormalities.

SUMMARY

A 23 month old boy with typical findings of oculodentodigital dysplasia syndrome was presented and the inheritance pattern of this syndrome was discussed.

ACKNOWLEDGEMENTS

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dactyly camptodactyly microcornea microphthal-
mus enamel hypoplasia

CASE REPORT

PROLONGED Q T INTERVAL AND CARDIAC SYNCOPES

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and Department of Paediatrics Central County Hospital Nykøbing Falster Denmark*

Cardiac syncopes in children due to malignant tachyarrhythmias are rare especially when no discernible organic heart disease is present (19)

A syndrome consisting of syncopal attacks congenital deaf mutism and Q T prolongation in the electrocardiogram was described by Jerrell & Lange Nielsen in 1957 (9) and then by several others (2 4 5 8 10-15)

Later syncopal attacks have been described in families with prolonged Q T interval but with normal hearing (1 6 7 12 16 18)

It is the aim of this paper to draw attention to this latter syndrome by reporting a case and the results of the family investigation

CASE REPORT

Boy born October 1967. He is the first of two children the other sibling being a 17 months younger brother. The pregnancy and delivery were normal and the birth weight was 3 200 g. Growth and development have been normal and he has had no childhood diseases.

At the age of 9 months he started having syncopal attacks. He usually started to scream, hid his head in his hands, became cyanotic on the lips and then fell unconscious to the ground. He had stiff limbs but no convulsions or incontinence. When he recovered after 1 minute he was lethargic and slept for about half an hour.

His attacks were sometimes provoked by excitement. When he had a fever he used to have several attacks at short intervals. In October 1969 he had had a total number of about a dozen attacks.

Between the age of 18 months and 2 years he was admitted twice to the paediatric department of his

regional hospital and once to the paediatric department of the university hospital.

Physical examination and chest roentgenogram were within normal limits. Repeated electroencephalograms were entirely normal. All laboratory tests including blood sugar, serum electrolytes, serum calcium, serum magnesium, serum uric acid and complete haematology were normal. The acid base state was normal. He had normal hearing.

A low pulse rate (of about 70 per minute) was noted at times and the electrocardiogram was abnormal: the T waves changed in configuration being negative at times and there was a constantly prolonged Q-T interval (Figs 1-2).

A 24 hours continuous ECG recording was obtained with telemetry and revealed no abnormal rhythm. No ECG was obtained during an attack. ECG during carotid sinus stimulation or while he was crying (performing a Valsalva maneuver) was unchanged.

A diagnosis of cardiac syncopes in association with the prolonged Q T syndrome was made and he was digitalised during his last admission at the age of 2 years. The Q T interval was hereby normalised (Fig 3). In the following 8 months he was doing well, having only two short lasting attacks when the medication was not given by mistake. The parents have been instructed in the techniques of resuscitation.

Family investigation

Both parents are healthy. The father was adopted and nothing is known about his family. In the mother's family several members had migraine and a sister of the mother the maternal grandmother's brother and one of his sons had spells of convulsions in childhood supposed to be febrile convulsions (Fig 4). The maternal grandmother's mother was said to have had fainting spells when she was young; she died forty years ago of unrelated disease.

There was no history of congenital deafness or sudden death in the family.

An electrocardiogram was obtained in 26 members

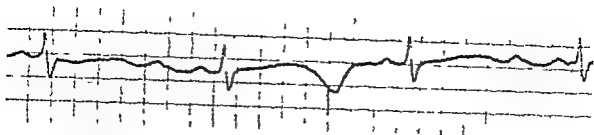


Fig 1 Electrocardiogram of our case (standard lead II) obtained in September 1969. The QT interval is prolonged (R-R 0.78 sec QT 0.58 sec. Upper normal limit 0.40 sec according to Ahmström et al. *Circulation*

1950). Paper speed 50 mm/sec; the distance between the distinct vertical lines being 0.10 sec.

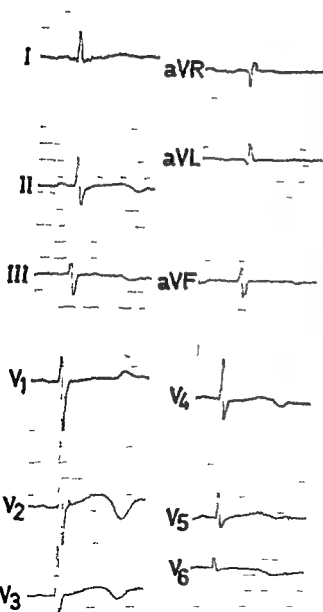


Fig 2 Electrocardiogram of our case obtained in September 1969. Note the bizarre T waves in the precordial leads.

of the family (Fig. 4) including all the mothers, siblings and their children and including those mentioned above with previous convulsions. A prolonged QT interval was found in the patient's younger brother (Fig. 5) and a borderline value in a cousin. Neither of them have had syncopal attacks. The maternal grandmother's father refused to have an ECG taken, but an ECG from a hospital admission in 1964 showed a slightly prolonged QT interval (R-R 0.75 sec QT 0.41 sec) the T waves were normal and he had never fainted.

DISCUSSION

The combination of prolonged QT interval and syncopal attacks is described with increasing frequency both in patients with congenital deafness and in patients with normal hearing. The number of reported cases is approaching fifty but there are probably several unrecognized patients who are treated for epileptic spells. Most of the cases have been described in members of the same family (1, 5, 6, 7, 9, 13, 16, 18) with autosomal dominant inheritance suggested for those with normal hearing (7) while autosomal recessive inheritance is claimed for those with congenital deafness (5).

In those cases in whom an electrocardiogram was obtained during an attack, the cardiac arrhythmia responsible for the syncope was shown to be ventricular fibrillation (2, 7, 8, 10, 16, 18).

The etiology of the prolonged QT interval is not clearly understood but is probably due to a metabolic abnormality in the myocardium which is not capable of normal repolarization (7). The mechanism by which the ventricular fibrillation may be precipitated has recently (7) been demonstrated to be 1) an increase in

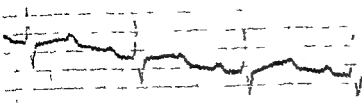


Fig 3 Electrocardiogram of our case (standard lead II) obtained in October 1969 after digitalisation. The QT interval is now normal.

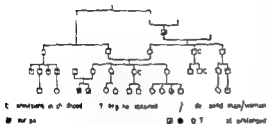


Fig 4 Pedigree of the family of our case. Electrocardiograms were obtained in all living persons not marked with ?

the systemic blood pressure (pressure induced extrasystoles) 2) sinus tachycardia (when the impulse reaches the ventricles still in a depolarized state) or 3) an extrasystole produced in the supernormal phase of repolarization (Romano-Ward syndrome (3, 17)).

The syncopal attacks start in infancy or childhood, the onset having been described between the age of 2 months and 12 years. The attacks are usually provoked by exertional or emotional strain.

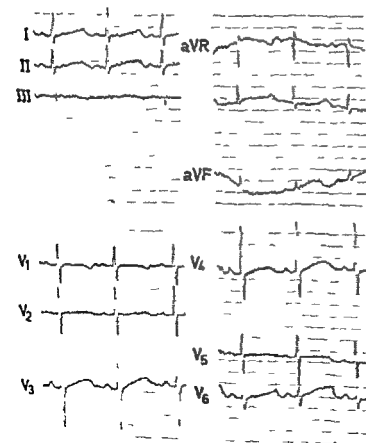


Fig 5 Electrocardiogram of our patient's younger brother obtained in October 1969. The QT interval is prolonged (RR 0.50 sec, QT 0.36 sec, upper normal limit 0.32 sec).

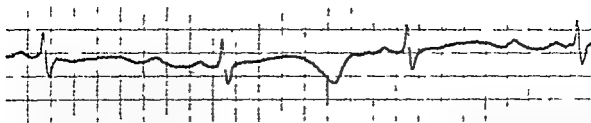


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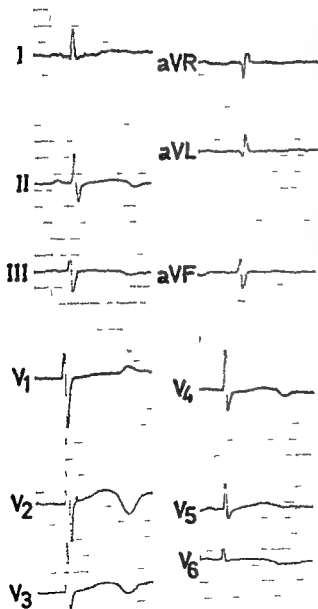


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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting Jan 14 1970

N J Brandt B Fris Hansen & J C Melchior
Diastrophic dwarfism

Two patients with diastrophic dwarfism were demonstrated

The first was born by natural delivery following a normal pregnancy. The birth weight was 3 000 g. At birth cleft palate and deformities of the extremities were demonstrated. On both hands the fifth finger was crooked with radial angulation of the terminal phalanx and the first finger was very short. Slight medial angulation of both femora, marked curvature of both legs and severe pes equinus on both sides were also present. The joints showed alternating contractures and hyperflexibility.

The second patient was born at term by breech delivery following a normal pregnancy. The birth weight was 2 800 g. At birth the appearance was dysmature and the following were present: large cephalhaematoma in the occipital region, cleft palate, short upper limbs, hyperextension of the knees with dislocation of the patellae, 60° posterior angulation of both tibiae and slight equino-varus deformity of the feet.

Hitherto over 20 cases of this syndrome have been described in the literature. The syndrome includes the following: (1) deformities of the extremities, (2) scoliosis, (3) cleft palate and (4) deformed external ears. The deformities of the extremities are particularly characteristic on account of the degree of twisting especially of the lower limbs. All limbs are short and podgy and the epiphyses are particularly deformed. The first metacarpal is practically in

variably short and frequently deformed. The disease is considered to be of autosomal recessive heredity.

In the cases described here the family histories were negative. All four parents had however been employed in departments of anaesthesia about the time of conception and the mothers had continued to work there during pregnancy.

Ole Ortvad Andersen & Knud E Petersen
An enuresis material from the Children's Hospital Fuglebakken

An account is given of a material of 135 children (76 boys and 59 girls) aged 4-15 years who were admitted on account of enuresis to the Children's Hospital Fuglebakken, Copenhagen.

In an attempt to obtain a more differentiated picture and thus a possibility for more rational therapy of this very heterogeneous group of patients the material was subdivided into five groups.

Group I: Children in whom the bladder function had probably matured late. These were selected according to the criteria of primary enuresis nocturna and as far as possible deep sleep. Further children who slept very deeply with secondary and possibly both nocturnal and diurnal enuresis were included in this group. None of these children exhibited the marked symptoms which were characteristic for Groups II and III. Forty-one per cent of

The prognosis is grave as more than one third of the reported cases have died during an attack. The danger of a fatal outcome seems to be diminished once the patients reach adulthood (10). Autopsy in a few cases conducted with special reference to the conducting system have shown consistent abnormalities (5).

Several antiarrhythmic drugs have been advocated as prophylactic treatment. Best effect has been reported with digitalis which shortens the Q-T interval (4, 10), and with beta blocking agents (7, 18). Other measures are restraint from overexertion and emotional stress and instruction of the relatives, neighbours, school teachers etc. in resuscitation technique.

As to our patient, there can be no doubt that he has cardiac syncope in association with prolonged Q-T interval though we have not recorded an ECG during an attack. He is doing well on treatment with digitalis but should his attacks persist, we will change the medication to a beta blocking agent. We have not been able to find any other member of the family with cardiac syncope though prolonged Q-T interval has been found in his brother and in a 5 year old ECG of his maternal grandfather's father.

An ECG should be obtained in any child with spells of unconsciousness; the diagnosis of prolonged Q-T interval is easily made and prophylactic antiarrhythmic treatment is important in view of the grave prognosis. Should the Q-T interval be normal the child may still have cardiac syncope and an exercise ECG should be obtained if possible (19).

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Group I: Children in whom the bladder function had probably matured late. These were selected according to the criteria of primary enuresis nocturna and as far as possible deep sleep. Further, children who slept very deeply with secondary and possibly both nocturnal and diurnal enuresis were included in this group. None of these children exhibited the marked symptoms which were characteristic for Groups II and III. Forty-one per cent of

the children mainly boys, were classified in this group

Group II Children with evidence of small bladders. These were selected on account of the symptoms of frequent micturition and urgency of micturition. These should electively have primary nocturnal enuresis. Children with secondary or possibly combined nocturnal and diurnal enuresis were included in this group provided they did not present the symptoms of Group III. Ten per cent of the children mainly girls were classified in this group.

Group III Children with emotional problems. Disturbances of behaviour or environmental factors which were considered to constitute emotional burdens were the decisive criteria for selection. The children in this group should have secondary enuresis but children with primary enuresis and pronounced behaviour disturbances were included. Forty per cent of the children mainly girls qualified for this group.

Group IV Children with malformations of the urinary tract or recurrent urinary tract infections. Six per cent of the children mainly girls were encountered in this group.

Group V Very immature children who were mentally retarded or psychoinfantile. Three per cent of the children could be placed in this group.

Knud E. Petersen & Ole Orsted Andersen
Treatment of nocturnal enuresis with imipramine and related preparations. A double-blind trial with a placebo

The mechanism of the effect of imipramine on enuresis has not yet been elucidated. In addition to its anti-depressive effect the preparation has also an anti-cholinergic effect. In order to investigate the mode of action the authors investigated imipramine together with imipramine N oxide which has the same anti-depressive effect but is without effect on the autonomic nervous system and emepromium, an anti-cholinergic preparation.

In an out-patient therapeutic trial the prep-

arations were administered together with a placebo in randomized order employing a double-blind technique to 69 children who had previously been admitted to the Children's Hospital Fuglebakken on account of enuresis and who were still wet at night. The distribution of the children in the enuresis groups was as described in the previous lecture. The dosage employed was 50 mg (corresponding to 1-2.5 mg/kg) administered 1 hour before bedtime. This dosage appears to be equally effective in all age groups from 4 to 15 years. Apart from tremor during imipramine therapy in 1 patient no side-effects necessitating withdrawal of treatment were registered.

Thirty per cent of the children became dry following treatment with imipramine for 2 weeks. Imipramine reduced the number of wet nights in the entire material to approximately 50% of the incidence in a corresponding placebo period. By contrast imipramine N oxide lowered the incidence only to 75% while emepromium had the same effect as a placebo. In assessment of the results the protracted effect of the preparations was taken into consideration. In certain children particularly those with presumed psychogenic causes (Group III) the incidence of enuresis could be reduced to 30%. The incidence increased slightly, but the effect was still very pronounced after treatment for 3 months with imipramine; spontaneous improvement being taken into consideration. Following cessation of treatment the incidence of enuresis increased to the previous level.

The anti-cholinergic effect is without significance. The psychic effect of imipramine is of significance in connection with enuresis possibly in combination with an effect on sleep.

O. Steinicke *Imipramine treatment of enuresis. A material from Christmas Seal Homes*

In order to assess the effect of imipramine (Toframil®) on enuresis all children admitted during a period of 1 year on account of this condition to Christmas Seal Homes were in-

cluded in a therapeutic trial with a placebo group. In addition the children were followed up 3-4 months after discharge.

A total of 225 children aged 4-14 years were treated. These children had enuresis more than thrice weekly before admission. Tofranil was administered to 110 and a placebo to 115 children. Treatment was administered for 60 days. Children aged 4-6 years received 10 mg, children 7-9 years 20 mg and children 10-14 years received 30 mg. The preparation was administered 1-2 hours before bedtime in cases of nocturnal enuresis and in diurnal enuresis in corresponding doses in the morning. Children with both nocturnal and diurnal enuresis received medication both morning and evening. In evaluating the results the latter group was assessed separately for both diurnal and nocturnal enuresis, 23 patients in the Tofranil group and 31 in the placebo group being involved. These cases were thus added to both the purely diurnal and the purely nocturnal groups.

Nocturnal enuresis comprised by far the largest group and in 104 cases treated with To-

framil good improvement was demonstrated i.e. the incidence of enuresis was reduced to less than one third of the original incidence in 36%. In comparison 107 cases were treated with the placebo and good improvement occurred in 20%.

On follow up investigation 3-4 months after the stay in the Christmas School Home good improvement had occurred in 29% of 94 patients treated with Tofranil as compared with 15% of 96 patients in the placebo group. Corresponding figures in patients with diurnal enuresis were good improvement in 52% of 29 patients treated with Tofranil and 28% of 39 patients treated with the placebo and on follow up examination 26% and 14% respectively.

Thus both during institutionalization and on follow up examination the results from Tofranil appear to have been somewhat better than from the placebo. The author considers therefore that Tofranil should be considered as a therapeutic possibility in enuresis although in slightly larger dosages than those employed here and only for 2-3 months.

Meeting Febr 11 1970

J. Hazhr & L. Bohn *Uricult*: A simple method of semi-quantitative culture from urine

Uricult is a semi-quantitative object glass method of demonstration of bacteriuria elaborated on the basis of the works of Naylor & Guttman in 1967. An object glass is covered on one side with meat extract peptone agar and on the other with MacConkey's agar. The glass is dipped into the specimen of urine and incubated at 35°C. On the following day the number of bacteria are assessed by comparison with the accompanying scale. Mid stream specimens of urine are employed or specimens obtained by means of corresponding technique. Uricult does not require refrigeration during transport.

In Glostrup Hospital (Department of Paediatrics and Central Laboratory) we have under-

taken a comparison between Uricult and ordinary quantitative culture according to the loop method. A total of 438 specimens were investigated and 121 of these were from children under the age of 15 years. The period of incubation was 18-20 hours. Significant growth was obtained in 213 specimens i.e. at least 100 000 bacteria per ml by both methods. In 12 cases lack of agreement was present as only one of the methods showed significant growth. When compared with the clinical findings and microscopic examination of the urine it appeared however that only two infections were not revealed by Uricult and similarly two infections employing the conventional method.

In 67 specimens with significant growth employing the loop method and Uricult at 35°C the Uricult method employed at room temperature

the children mainly boys, were classified in this group

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The anti-cholinergic effect is without significance. The psychic effect of imipramine is of significance in connection with enuresis possibly in combination with an effect on sleep.

O. Steinicke. Imipramine treatment of enuresis. A material from Christmas Seal Homes

In order to assess the effect of imipramine (Tofranil®) on enuresis all children admitted during a period of 1 year on account of this condition to Christmas Seal Homes were in-

slight webbing between the fingers of the left hand. The shoulder and elbow joints appeared normal and there was no evidence of pareses. The hip joints were freely mobile and there were slight contractures in the knee joints.

The feet were deformed on both sides but more so on the left side where the musculature of the leg was atrophic and a pronounced club-foot deformity was present. On the right side there was complete dislocation of both the talo-crural and subtalar joints.

The oesophagus would not permit passage of a probe and radiography confirmed the suspicion of atresia of the oesophagus. No clinical evidence of cardiac or pulmonary disease was present. The ordinary laboratory investigations for blood sugar, haemoglobin, serum calcium and microscopic examination of the urine revealed normal findings. On account of the low values for oestradiol excretion found in the maternal urine, the infant's urine was investigated for fractionated 17 ketosteroids and values which were probably normal for the neonatal period were found.

The infant died at the age of 93 hours. Autopsy revealed:

Increased quantities of cerebro-spinal fluid and agenesis of the corpus callosum. Atresia of the oesophagus with fistula formation between the distal oesophageal segment and the trachea. The left lung showed complete absence of lobulation. No cardiac defects were present. The gall bladder and the cystic duct were absent but a normal papilla of Vater was present. A probe could be passed for some centimetres into the common bile duct which appeared to disappear into the liver as a fibrous band. No deformities were encountered in the urinary tracts and the vagina, uterus, salpinges and ovaries were normal for the age.

The suprarenal glands were rather small but otherwise normal. Microscopic examination of the kidneys, liver and lungs showed normal conditions.

Chromosome investigation undertaken on cultured lymphocytes showed triploidy in all the 43 cells examined. The sex chromosomes

were XXX. Autoradiography revealed that there were two late-synthesizing X chromosomes in 12 out of 22 cells examined and in nine cells one late synthesizing X was found.

Blood type investigation suggested that it was most probable that the patient had received two haploid chromosomes from the mother. The results suggested that the patient had received both of the mother's rhesus alleles. The child showed double-dose effect to anti M while the father had type N and the mother type M.

Triploidy has previously only been described in two live born infants while it occurs relatively frequently in spontaneous abortions where it is the third most common chromosome abnormality.

Karen Thomsen. Diabetic coma without ketonuria

Hyperosmolar diabetic coma without ketosis is rare in childhood. In June 1969 a male infant aged 9 months was admitted to the Paediatric Department, Glostrup Hospital with this condition. He had high fever, was dehydrated, confused but not unconscious and pneumonia was present. The blood sugar was 1232 mg/100 ml and the serum osmolality was calculated as 359 mOsm/kg H₂O. The standard bicarbonate was 12 mEq/l. The urine remained free of acetone until 8 days after admission when the infant again developed high blood sugar and began to form acetone.

The child was treated with insulin in relatively small repeated doses and intravenous fluid in the form of isotonic saline, bicarbonate and plasma. On this treatment he recovered slowly and could be discharged later in good condition with 0.15 ml Insulin Rapi-tard daily.

The syndrome of hyperosmolar diabetic coma without ketonuria is characterized by severe hyperglycaemia, hyperosmolality and hypotonic dehydration and the absence of ketosis. Some cases but not all have lactic acidosis. In this case the serum lactic acid was not determined. In those cases that have been pub-

(20–23°C) rendered six false negative results but no false positive results. If 10 000 bacteria per ml had been employed as the limit of significant bacterium, only one false negative result would have been found. The material does not permit conclusions to be drawn regarding the employability of Uricult at room temperature.

Uricult provided equally reliable results as ordinary culture. The method cannot replace microscopic examination of urine but provides a suitable supplement to this.

§ Sparrevoth & N. J. B. Christiansen. A case of congenital myxoedema diagnosed on the second day of life by microanalysis of serum thyroxine.

The patient was a male infant, the second of two siblings. He was transferred to the Paediatric Department in Glostrup Hospital immediately after birth on account of a swelling as large as a fist on the anterior aspect of the neck and respiratory distress. The birth weight was 3 400 g, delivery occurred at term and the condition at birth was good in other respects. The swelling was demonstrated to be a large goitre of cystic character. The serum thyroxine on the second day of life was 8.9 µg/100 ml and 2.3 µg/100 ml on the fifth day of life.

During the first 5 months of pregnancy the mother had consumed some special energy tablets and in this manner she had received approximately 7 g lithium and approximately 13 g potassium iodide. The infant was given Eltroxin® therapy in a dosage of 0.1 mg daily and the goitre had disappeared entirely by the third week of life. Radiographic examination revealed that Belding's centres of ossification were absent at birth. Protein bound iodine on the second day was 5.7 µg/100 ml.

When the boy was 6 months of age Eltroxin treatment was withdrawn and follow up and control of serum thyroxine have since shown normal conditions. No known cases of thyroid disease were present in the family. The pronounced congenital goitre and myxoedema were

probably due to abnormal sensitivity to iodine.

This case history, similarly, emphasizes the great value of microanalysis for determination of serum thyroxine. Only small quantities of blood are required (100 µl serum) and the analysis has the great advantage that contamination with iodine is of no consequence (K. Siersbæk Nielsen & J. Møhlholm Hansen, *Acta Paediatr Scand* 56 141 1967).

S. Sparrevoth, M. Mikkelsen, E. Niebuhr & K. Henningsen. A live born patient with triploidy.

A newly born female infant was transferred from the Maternity Department to the Department for Neonates in Glostrup Hospital. The child was the second of two siblings. Her brother, aged 1 year, was healthy. The mother had had an abortion in the third month in 1967. Apart from this there were no known abortions or children with malformations in the family. The pregnancy had been completely uncomplicated with no signs of infection and the mother had not taken any form of medication nor contraceptive pills. At 3 weeks and 11 days prior to the delivery oestriol excretion was low but the foetal movements and heart sounds were normal. The foetus appeared to be abnormally small and 3 weeks before term caesarean section was performed. The amniotic fluid was greenish, the infant limp with a low Apgar scoring, birth weight 1 600 g, length 40 cm and circumference of head 33.5 cm. The infant appeared very dysmature and had numerous malformations. The head was strikingly large although not hydrocephalic, the ears small and deformed protruding and slightly low set, there was slight micrognathia, bilateral blepharophimosis but the bulbs were normal. The upper lip was rather long and thick. The left hand was slightly deformed as if resulting from intrauterine pressure. The hands were peculiar with long fingers of arachnodactyly type and the origins of the three radial fingers were more distal from the hand than the two ulnar fingers. There was

face. The lips became cyanotic and shortly afterwards he fell lost consciousness and had rattling respiration but no spasms. After a few minutes he recovered consciousness and then slept for about half an hour after which he appeared completely normal again.

The child was at first suspected of having epilepsy but all relevant investigations for epilepsy had negative results.

On the other hand after the attacks the electrocardiogram was abnormal with very varying T waves which gradually become normalized while prolongation of the Q-T interval persisted. All electrolyte determinations showed normal findings. Treatment with Digoxin was commenced. The Q-T interval returned to normal and the boy has been free from attacks for over 6 months.

On investigation of the patient's family a prolonged Q-T interval was demonstrated in his brother who is 17 months younger. This boy has hitherto not had any cardiac syncope.

H T Lund, Ib Transbøl & Ib Hornum. *Hypercalcaemic sarcoidosis. Report of a case in a girl aged 13 years.*

An unusual case of hypercalcaemia in a girl aged 13 years is described. Her symptoms which consisted of fatigue, loss of weight, polyuria and thirst could all be attributed to the findings of lowered renal function and hypercalcaemia. Investigation of the calcium metabolism revealed excessive hypercalcaemia and metastatic calcification of the cornea (split lamp examination) and kidneys (renal biopsy). Tubular reabsorption of calcium (TRCa) and the cortisone test both suggested that the cause of the hypercalcaemia was non-parathyroid.

The Mantoux reaction was negative despite previous BCG vaccination. The raised sedimentation rate and serum gammaglobulin and a muscle biopsy showing chronic inflammatory changes and multinuclear giant cells were the positive findings which suggested sarcoidosis as the only probable diagnosis.

The patient was treated with prednisone after which the calcium metabolism and the renal function returned to normal.

M Fjord Christensen & A J Therkelsen. *A case of a XXXYY chromosome anomaly in a boy aged 2 1/2 years.*

The sex chromosome anomaly 49 XXXXY has been described in 42 patients since 1960. A clinical picture with oligophrenia, certain mongoloid features, hypogonadism and slight skeletal malformations has been found to be typical for the anomaly.

A new case of the 49 XXXXY anomaly in a boy aged 2 1/2 years with the typical clinical picture is presented.

Xg blood type determinations in the patient and his parents revealed that all four X chromosomes were maternal which suggests that the anomaly has developed by non-disjunction in each of the egg cell's two meiotic divisions.

The finding of a diabetic glucose tolerance curve is discussed in relation to the increased incidence of diabetes mellitus in patients with Klinefelter's syndrome and in patients with Turner's syndrome. The genetic imbalance with either too many or too few X chromosomes may predispose to diabetes mellitus.

Sug Sparrevojn. *Four patients with saccharose malabsorption.*

Meeting May 13, 1970

Bent Friis Hansen. *Directions for oxygen therapy in newly born infants* (Published in *Medicinsk Arbejde XIII* Munksgaard 1970 pp 167-187).

H H Sørensen. *Renal fibrosis*

II Djernes. *Regional enteritis (Crohn's disease) in childhood.*

Regional enteritis is a rare condition in childhood which is perhaps reported too seldom. The symptoms are frequently generalized and

lished, the mortality was 50%. Early diagnosis and intensive fluid and insulin therapy are important in order to lower the mortality.

J Høhr & S Sparrevoth *Epididymitis in children*

Acute epididymitis is considered to be very rare prior to puberty. The clinical diagnosis may be extremely difficult and as a rule emergency operation is indicated in order to exclude torsion of the testis. In the majority of cases the etiology is unknown but in some cases urinary tract infection appears to be the cause.

In the Paediatric Department in Glostrup Hospital 6 cases of epididymitis have been admitted in the course of the past 4 years. Two of these developed in connection with Schönlein-Henoch's purpura. This complication has been mentioned only once previously in the literature. One patient had suppurative epididymitis. Another case occurred as a complication of orchidopexy while no explanation

could be discovered for the 2 remaining cases. In all of the cases the diagnosis was verified by operation. None of the patients had had urinary infection prior to, during or after admission. One child had a double renal system without other malformation and the remainder were all demonstrated by urography to have normal urinary tracts. Three patients including the two with Schönlein-Henoch's purpura, had thrombocytosis of long duration.

Biopsy from the epididymis from the 2 patients with Schönlein-Henoch's purpura showed pronounced necrosis of the vascular walls while the epithelium in the ductuli was normal. The picture differed distinctly from the biopsy findings in the 2 children with idiopathic epididymitis where the changes were localized to the ductuli with destruction of the epithelium and inflammatory infiltration while the vessels were normal.

Follow up examination from 6 months to 3 years after admission revealed normal findings on palpation of the scrotum in all the cases.

Meeting March 11 1970

Discussion concerning the expansion of paediatrics in the future hospital services in Denmark.

Meeting April 8 1970

Jørgen Kringelbach & Alf Wennevold *Prolonged Q-T interval and cardiac syncope (Ward's syndrome)*

In 1957 Jervel & Linge described a syndrome in infants with congenital deafness consisting of cardiac syncope and prolonged Q-T interval accompanied by sudden death. In subsequent years, this surdo-cardiac syndrome was described by various other authors from many different countries. In 1963-64 reports of similar cases in children with normal hearing were published inter alia from Italy and Ireland (Ward).

A total of over 50 cases of the combination

prolonged Q-T and cardiac syncope have now been described.

The first recognized case in Denmark is presented.

The patient is a boy now aged 2 1/2 years. He is the first of two siblings born to healthy parents; the hearing is normal; there is no known familial deafness nor predisposition to epilepsy; the pregnancy and delivery were normal and the infant did not have asphyxia.

From the age of 14 months the boy had had a total of ten attacks. These commenced suddenly with violent screaming as if in pain. The hands were clenched and held in front of the

face. The lips became cyanotic and shortly afterwards he fell lost consciousness and had rattling respiration but no spasms. After a few minutes he recovered consciousness and then slept for about half an hour after which he appeared completely normal again.

The child was at first suspected of having epilepsy but all relevant investigations for epilepsy had negative results.

On the other hand after the attacks the electrocardiogram was abnormal with very varying T waves which gradually become normalized while prolongation of the Q-T interval persisted. All electrolyte determinations showed normal findings. Treatment with Digoxin was commenced. The Q-T interval returned to normal and the boy has been free from attacks for over 6 months.

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Meeting May 13 1970

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H H Sedorff. *Retrolental fibroplasia*

B Djernes. *Regional enteritis (Crohn's disease) in childhood.*

Regional enteritis is a rare condition in childhood which is perhaps reported too seldom. The symptoms are frequently generalized and

insidious Meticulous examination of extensive materials has revealed that, in 14% of the cases diagnosed in adult life, the first symptoms had occurred prior to the age of 15 years

Two cases are presented One was a boy aged 12 $\frac{1}{2}$ years On admission the symptoms had been present for 2 months in the form of non specific systemic symptoms anaemia, raised sedimentation rate and only slight and transient gastro intestinal symptoms The height was below normal and the state of nutrition so far below average that a malignant condition was immediately suspected The diagnosis was established by means of contrast enemata in the colon undertaken on account of slightly abnormal duodenal course observed following a barium meal and partly because of demonstration of port-wine discoloration of the urine on standing which proved to be due to indicanuria On laparotomy, severe changes in the distal third of the ileum the entire ascending colon and the greater part of the transverse colon macroscopically and microscopically compatible with Crohn's disease, were revealed Treatment in the form of resection of the colon with ileo sigmoidostomy and resection of the section of the ileum affected combined with postoperative medical therapy with diet and Salazopyrin resulted in complete recovery increase in weight and increase in height

The other case was a boy aged 13 years who was transferred from an ear nose and throat department where he had been admitted in view of tonsillectomy on account of tonsillar hypertrophy, anaemia and raised sedimentation rate but where he had developed diarrhoea Further investigation of the case history revealed periods with frequent loose motions during the preceding months On admission the patient was subfebrile The height was normal for the age but the state of nutrition was definitely below normal He did not appear however to suffer from chronic illness The subsequent course was dominated by intermittent fever the general condition deteriorated gradually and an abscess developed at the anus and proved

difficult to treat Although radiographic examination of the alimentary canal including investigation of the appendix by Chromos method and repeated barium enemata did not reveal any abnormalities apart from a para rectal abscess explorative laparotomy revealed pronounced changes in the distal third of the ileum the macroscopic appearance of which corresponded to Crohn's disease This diagnosis could also be confirmed by biopsy from the section of intestine involved As the remainder of the alimentary canal appeared to be entirely normal no resection was undertaken and instead the patient was treated postoperatively with diet and steroids and, later, with diet and Salazopyrin² which resulted in general recovery and thriving The subsequent course was complicated 6 months after the laparotomy by obstruction due to a band of adhesions which was treated surgically by intestinal resection On this occasion, there was no evidence of active enterocolitis and since then the course has been uncomplicated and favourable

Neither of these patients presented any particularly alarming abdominal symptoms One of them had practically normal motions and both had normal or scarcely characteristic gastro intestinal passage on radiographic examination and nevertheless, they presented severe intestinal changes on explorative laparotomy The discoloration of the urine observed in the first patient, when proved to be due to indican suggested a pathological condition in the small intestine This does not appear to have been described previously although it is recognized that patients with this condition and a number of other conditions with increased bacterial growth in the small intestine and a number of conditions with malabsorption have excretion of indican in the urine Finally in the same patient it was observed that the Moro reaction became negative during the course of the disease despite previous BCG vaccination Similar observations have been made by other authors

N J Brandt

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pp Jonny Reads Inc St Petersburg Florida 1970
US \$7.50

insidious Meticulous examination of extensive materials has revealed that, in 14% of the cases diagnosed in adult life the first symptoms had occurred prior to the age of 15 years

Two cases are presented One was a boy aged 12 $\frac{1}{2}$ years On admission the symptoms had been present for 2 months in the form of non specific systemic symptoms, anaemia raised sedimentation rate and only slight and transient gastro intestinal symptoms The height was below normal and the state of nutrition so far below average that a malignant condition was immediately suspected The diagnosis was established by means of contrast enemata in the colon undertaken on account of slightly abnormal duodenal course observed following a barium meal and partly because of demonstration of port wine discoloration of the urine on standing which proved to be due to indicanuria On laparotomy, severe changes in the distal third of the ileum, the entire ascending colon and the greater part of the transverse colon macroscopically and microscopically compatible with Crohn's disease were revealed Treatment in the form of resection of the colon with ileo sigmoidostomy and resection of the section of the ileum affected combined with postoperative medical therapy with diet and Salazopyrin resulted in complete recovery increase in weight and increase in height

The other case was a boy aged 13 years who was transferred from an ear, nose and throat department where he had been admitted in view of tonsillectomy on account of tonsillar hypertrophy anemia and raised sedimentation rate but where he had developed diarrhoea Further investigation of the case history revealed periods with frequent loose motions during the preceding months On admission the patient was subfebrile The height was normal for the age but the state of nutrition was definitely below normal He did not appear however to suffer from chronic illness The subsequent course was dominated by intermittent fever the general condition deteriorated gradually and an abscess developed at the anus and proved

difficult to treat Although radiographic examination of the alimentary canal including investigation of the appendix by Chrom method and repeated barium enemata, did not reveal any abnormalities apart from a para rectal abscess, explorative laparotomy revealed pronounced changes in the distal third of the ileum the macroscopic appearance of which corresponded to Crohn's disease This diagnosis could also be confirmed by biopsy from the section of intestine involved As the remainder of the alimentary canal appeared to be entirely normal, no resection was undertaken and instead the patient was treated postoperatively with diet and steroids and, later, with diet and Salazopyrin® which resulted in general recovery and thriving The subsequent course was complicated 6 months after the laparotomy by obstruction due to a band of adhesions which was treated surgically by intestinal resection (On this occasion there was no evidence of active enterocolitis and since then the course has been uncomplicated and favourable

Neither of these patients presented any particularly alarming abdominal symptoms One of them had practically normal motions and both had normal or scarcely characteristic gastro intestinal passage on radiographic examination and, nevertheless, they presented severe intestinal changes on explorative laparotomy The discoloration of the urine observed in the first patient, when proved to be due to indican suggested a pathological condition in the small intestine This does not appear to have been described previously although it is recognized that patients with this condition and a number of other conditions with increased bacterial growth in the small intestine and a number of conditions with malabsorption have excretion of indican in the urine Finally, in the same patient it was observed that the Moro reaction became negative during the course of the disease despite previous BCG vaccination Similar observations have been made by other authors

N J Brandt

NEW BOOKS RECEIVED

- J Huter & B Zehentbauer *Übergang von Medikamenten in Muttermilch und Nebenwirkungen beim gestillten Kind* 80 pp Georg Thieme Verlag Stuttgart 1970 DM 22—
- K. Decker & H Backmund *Pädiatrische Neuroradiologie* 193 pp illus Georg Thieme Verlag Stuttgart 1970 DM 79—
- J L Melnick (ed) *Progress in Medical Virology* Vol 17 357 pp illus S Karger AG Basel München, Paris and New York 1970 sFr 72— US \$17.30
- D R Laurence (ed) *Drugs Development and use* British Medical Bulletin Vol 26 1970 London £2.
- R Burkhardt *FarbAtlas der klinischen Histopathologie von Knochenmark und Knochen* 115 pp illus Springer Verlag Berlin Heidelberg and New York 1970 DM 748—
- F C Fraser & V A McKusick (eds) *Congenital Malformations Proceedings 3rd International Conference on Congenital Malformation* The Hague 1969 466 pp illus Excerpta Medica Amsterdam 1970 US \$ 7.50
- W Ludwig *Das Rechts Links Problem in Tierreich und beim Menschen* 496 pp illus Springer Verlag Berlin Heidelberg and New York 1970 Price not given
- G T Pack & A H Islami (eds) *Tumors of the liver Recent Results in Cancer Research* Vol 26 304 pp illus Springer Verlag Berlin Heidelberg and New York 1970 DM 56—
- R W McCammon *Human Growth and Development* 295 pp Charles C Thomas Publ Springfield Ill 1970 US \$9.00
- Th Ehrenpreis *Hirschsprungs disease* 175 pp illus Year Book Medical Publ Inc Chicago 1970 US \$13.50
- R E Gross *An atlas of children's surgery* 191 pp illus W B Saunders Co Ltd London 1970 £8 1s 6d
- M Gunther *Infant feeding* 114 pp Methuen & Co Ltd London 1970 25s
- R R Limner *Sex and the unborn child* 229 pp., The Julian Press Inc Publ., New York 1969 US \$6.95
- Perinatal factors affecting human development* Pan American Health Organization Scientific Publication No 185 Washington 1970
- H J Kaufmann (ed) *Progress in pediatric radiology* Vol 3 *Genito urinary tract* 381 pp illus Karger Basel München London Paris New York and Sydney 1970 DM 96—
- R Debre & J Celers (eds) *Clinical virology* 871 pp illus W B Saunders Co Philadelphia London and Toronto 1970 £16 3s
- M Bohman *Adopted Children and their families* 239 pp Proprius Stockholm 1970 Sw kr 45—
- L Sachs *Statistische Methoden Ein Soforhelfer* 103 pp Springer Verlag Berlin Heidelberg and New York 1970 DM 8.80
- J Dubois *Etude de l'équilibre hydro-électrolytique du tissu musculaire strié chez l'enfant* 162 pp illus Editions Arsacia S A Bruxelles 1970 320 F B
- G E W Wolstenholme & J Knight (eds) *Sensorial hearing loss* A Ciba Foundation Symposium 358 pp J & A Churchill Lt London 1970 80s
- J M Berg ■ D McCreary M A C Ridler & G F Smith *The de Lange Syndrome* Institute for Research into Mental Retardation Monograph No 2 127 pp illus Pergamon Press Ltd Oxford 1970 63s
- R C Wunderlich *Kids Brains and Learning* 534 pp Jonny Reads Inc St Petersburg Florida 1970 US \$7.50

BOOK REVIEWS

C C de Silva & N G Baptist *Tropical nutritional disorders of infants and children* Thomas Springfield Ill 1969 226 pp US \$11.50

This book is a monograph of American Lectures in Living Chemistry. The aim with this kind of book is to advance the newer knowledge of chemical medicine in the course of clinical practice. The interdependence of chemistry and medicine is so great that physicians are turning to chemistry and chemists to medicine in order to understand the underlying basis of life process in health and disease. Of the authors Silva is the pediatrician and Baptist the biochemist and both are from Ceylon.

The clinical and the biochemical aspects of the concept of growth and failure of growth are first discussed and the reader is given a few surveyable facts about several subjects. The authors do usually not comment on these facts but they give many references for further studies. Following the chapters on growth there are five chapters dealing with carbohydrates, lipids, proteins, vitamins and minerals. The emphasis is laid on the connection between these food factors and protein-calorie malnutrition (PCM). The main part is about proteins and there is also a short survey of different individual amino acids in PCM. One third of the whole book is about vitamins and the different deficiency syndromes. The authors write very detailed and this reflects the importance of these diseases in India and Ceylon in contrast to many other tropical countries e.g. East Africa. Vitamin B₁₂ deficiency in infants probably caused by reduction of the vitamin in breastmilk is described. The infants were very apathetic, had a lemon yellow colour with symmetrical hyperpigmented urticaric patches. They had involuntary movements of head, trunk and limbs and the tongue was

constantly protruded. Arms and legs showed jerky movements and all had megaloblastic anemia. The importance of giving potassium and magnesium in the treatment of PCM is stressed in the chapter about erythropoiesis and anemia. There are many references, but almost all are from years before 1967.

For the pediatrician interested in nutrition this book gives a good introduction to the biochemical basis and the clinical interpretation of many common tropical disorders of infants and children.

Bo Pallin

P F Bray *Neurology in Pediatrics* Year Book Medical Publishers Inc Chicago Ill 1969 514 pp illus. US \$23.50

This textbook in pediatric neurology is divided in two parts. The first dealing with differential diagnoses according to signs and symptoms, the second part giving more conventional description of various diseases. One chapter describes the laboratory aids and their value in clinical work and another deals with practical problems in acute situations. The tables are easy read and the illustrations instructive and mostly of good quality.

Pediatric neurology is however a very vast subject and some parts of the book are rather short. However a rather comprehensive literature list is given for the reader wanting further details. In spite of being superficial in some topics the book gives a good survey of pediatric neurology. It is quite useful as a reference book as well as for postgraduate studies.

Ingrid Bjerre

ANNOUNCEMENT

The 1971 Annual Meetings of The American Pediatric Society, Inc. and The Society for Pediatric Research will be held at Traymore Hotel Atlantic City, New Jersey.

Wednesday April 28 8:30 p.m. *Does comprehensive care make a difference?* A symposium sponsored by the American Pediatric Society, the Society for Pediatric Research and the Ambulatory Pediatric Society.

Thursday April 29 *Plenary Session* American Pediatric Society.

Friday April 30 *Subspecialties Session* The American Pediatric Society and The Society for Pediatric Research.

Saturday May 1 *Plenary Session* Society for Pediatric Research.

For information write to Charles D. Cook, M.D. (Secretary, American Pediatric Society) 333 Cedar Street, New Haven, Connecticut 06510 or Robert E. Greenberg, M.D. (Secretary, Society for Pediatric Research) 12012 Compton Avenue, Los Angeles, California 90059.

IMMUNOFLUORESCENT AND MORPHOLOGICAL STUDIES IN CONGENITAL NEPHROTIC SYNDROME¹

JUHANI RAPOLA and ERKKI SAVILAHTI

From the Children's Hospital University of Helsinki Helsinki Finland

The clinical course morphology and mode of inheritance of congenital nephrotic syndrome (CN) has been extensively studied and reviewed recently (8-22). The pathogenesis of the disease however has remained unsettled. Kousvalainen (12) showed immunoglobulins attached to the glomeruli of 5 autopsy specimens and in one biopsy specimen using immunofluorescent techniques. Similar results were obtained by Lange et al (14-15) and Kobayashi (11) in four additional cases. These latter authors also found binding of components of complement in their studies.

These results have been challenged by Hoyer et al (10). In studies of two monozygotic twins with characteristic CN they could not show IgG or β 2C globulins in biopsy specimens taken at 6 weeks and 2 months of age respectively. In the subsequent autopsy specimen of one of their patients at the age of 21 months they found slight to moderate staining for IgG and β 2C proteins in most of the glomeruli in a local and uneven fashion. They concluded that in their cases immune mechanisms are not primary in the pathogenesis of the disease.

Recent attempts to improve the invariably fatal outcome of CN patients by kidney transplantation (9) has provided us ample material to reinvestigate the morphologic and possible

immunopathologic changes in fresh CN kidneys.

CASE REPORTS

Case 1

This boy was the first child in a family with no definite family history of congenital nephrosis. However on the mother's side perinatal unexplained deaths had occurred in earlier generations. The pregnancy was uneventful, syphilitic serology during pregnancy was negative. The child was born after 38 weeks gestation and his birth weight was 3200 g and the weight of placenta was 1070 g. Because of the large placenta CN was suspected immediately after birth and heavy proteinuria (1500 mg/100 ml) was noted. At the age of 1 month the boy was transferred to our hospital. At that time he was oedematous especially in the eyelids and the scrotum. The abdomen was enlarged. Urinary protein was 520 mg/100 ml. Total serum protein was 3.1 g/100 ml. Albumin was 0.4 g/100 ml, α 2 macroglobulin 1.57 g/100 ml and gammaglobulin 0.29 g/100 ml in cellulose acetate electrophoresis. In immunoelectrophoresis the same changes were noted and in addition elevated IgM was seen. Serum cholesterol and triglycerides were elevated 374 mg/100 ml and 115 mM/l respectively. Angiography of the inferior vena showed it to be patent.

Nephrectomy was performed at the age of 3 months in order to perform kidney transplantation.

Case 2

This boy was the second child of parents with no family history of CN. Their first child was in good health. Pregnancy was normal and serological tests for syphilis during pregnancy were negative. The child was born after 37 weeks gestation, his birth weight was 2650 g and the weight of placenta was 1000 g. He was admitted to our hospital at the age of 2 weeks because of cyanosis and vomiting. At that time he was oedematous especially in the eye

¹This study is published in honor of Professor Dr med. H. H. Schäfer Hamburg at his 60th birthday.

lids. The abdomen was enlarged but no ascites was seen on an X-ray. Urinary protein was 210 mg/100 ml. Serum protein concentration was 3.9 g/100 ml. In cellulose acetate membrane electrophoresis albumin was 0.5 g/100 ml, α_2 macroglobulin 2.1 g/100 ml and gammaglobulin 0.1 g/100 ml. The same changes were seen in immunoelectrophoresis and in addition elevated IgM was seen and it was quantitatively 170 mg/100 ml. Cholesterol was 850 mg/100 ml. At the age of 6 months angiography of the inferior vena showed it to be patent. He was treated several times in hospital and nephrectomy was done at the age of 1 year 11 months in preparation for kidney transplantation.

MATERIALS AND METHODS

Whole kidneys were obtained immediately after surgical nephrectomy. Pieces of the tissue for light microscopy, electron microscopy and immunofluorescent microscopy were processed immediately and the remainder was frozen and stored at -20°C .

Light microscopy. Several 3 mm thick pieces of tissue consisting of both cortex and medulla were fixed in Bouin's fluid and paraffin sections of 2–3 μm were stained with hematoxylin and eosin, van Gieson's PAS, Mallory's trichrome and Gomori's periodic acid silver methenamine.

Electron microscopy. Several 1 mm pieces of cortical tissue were fixed in 2.5% phosphate buffered glutaraldehyde at pH 6.8, postfixed in 1% phosphate buffered osmium tetroxide at pH 6.8, dehydrated and embedded in Epon 812. 1 μm plastic sections were stained with 1% aqueous toluidine blue for orientation. Thin sections were stained with uranyl acetate and lead citrate. Three mature glomeruli from both cases were studied in a Zeiss EM 9A electron microscope.

Fluorescent microscopy. Commercial fluorescein isothiocyanate (FITC) labelled goat antisera to human IgG, IgM and IgA (Hyland Laboratories Inc., Calif., USA) were used. Rabbit antiserum to human β_2 globulin was prepared according to Stratton (24) and labelled with FITC (4/18). Fluorescein/protein ratio was 2.7 (26). In blocking controls commercial unlabelled goat antisera to human IgG (Munn research laboratories Inc., N.Y., USA), IgM (Mann) and IgA (Hyland) were used and the unlabelled antiserum to β_2 globulin was the same as that used for preparation of the FITC conjugate. Monospecificity of the antisera was checked by immunoelectrophoresis and double diffusion micromethod (3).

For direct immunofluorescent study small pieces of kidneys consisting both of cortical and medullary tissue were frozen with solid carbon dioxide. Sections of 5 μm were cut in a cryostat microtome at -20°C , picked up on microscope slides and dried at room temperature for 30 min. All subsequent procedures were performed at room temperature. Sections were washed for 4 hours in phosphate buffered isotonic saline, pH 7.2 (PBS), fixed in ethanol, methanol and chloroform (1:1:1 mixture) for 30 min

and washed in three changes of PBS 10 min in each. Excess PBS was drained off and the sections were overlaid with FITC conjugates. They were incubated in a humid box for 1 hour and washed in three changes of PBS 10 min each. The slides were swept dry under the sections mounted in PBS buffered glycerol (1:10) under coverslips and examined immediately.

HB 200 mercury vapour lamp was used as a light source. UG 5/1 mm and BG 12/1 mm excitation filters and a K 460 blocking filter were used for examination and photography in colour on Kodak Ektachrome daylight type film. For black and white photography Kodak Tri-X film was used together with a BG 12/5 mm excitation filter in combination with a K 530 blocking filter. A Leitz Ortholux microscope with cardoid dark field condensers was employed in these studies.

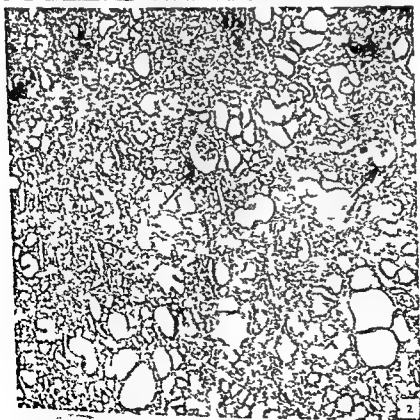
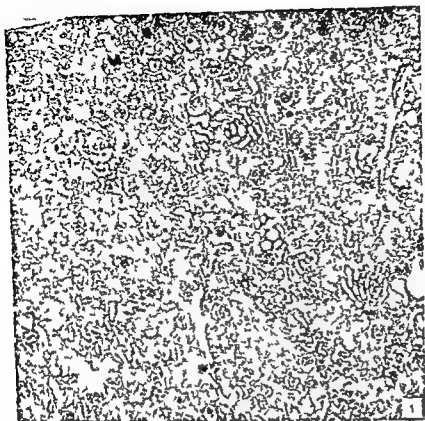
FITC conjugated antisera were diluted to a point giving bright specific fluorescence with minimal background on kidney biopsies taken from various glomerulonephritic disorders and processed similarly. In these previous studies a distinct linear or granular fluorescence along the glomerular capillary basement membrane was discerned depending on the character of the disorder. The specificity of staining was controlled by blocking procedures with unlabelled antisera causing clear diminution of the staining.

Kidney for indirect immunofluorescent study was obtained at surgical biopsy. It proved to be morphologically normal and negative on direct immunofluorescent studies. Tissue sections were fixed either in either alcohol for 10 min each (16) or processed in direct immunofluorescent staining. When the excess PBS was wiped off the sections were covered with eluate from nephrotic kidneys (see following paragraph). Slides were incubated in a humid box for one hour, washed in two changes of PBS for 10 min, wiped dry and covered with FITC conjugates. Antihuman IgG, IgM and IgA FITC conjugates were used in serial dilutions (1:2–1:32). After incubation for 1 hour sections were washed and processed as described before. A similar eluate from a normal kidney obtained at autopsy and serum IgG (1) served as a control.

Elution experiments. Half of one kidney of both cases and half of a morphologically normal control kidney obtained at autopsy 12 hours after death were processed according to the method of Lerner et al. (16) with slight modifications.

Fig 1 Kidney cortex from the case 1. Most glomeruli at the low magnification are relatively normal and dilatation of tubuli = mild and patchy. H & E $\times 60$.

Fig 2 Kidney from case 2. Glomeruli with condensed capillary tuft and widened urinary space (arrows) are common. Extensive tubular dilatation with some tubules containing casts is prominent. H & E $\times 60$.



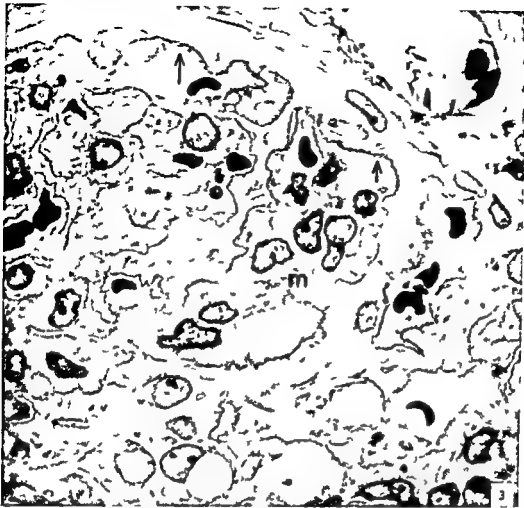


Fig 3 Portion of a glomerulus from case 2. Widening of the mesangial area (m) with hyperplasia of mesangial cells is evident. Peripheral capillary loops

are widely open and the basement membranes (arrows) thin. Epon Toluidine blue $\times 1400$.

The fragmented kidney pieces were washed for a short time and homogenized with Ultra Turrax for six times 5 sec at 0°C. Homogenates were suspended in PBS pH 7.2 and centrifuged at 23 000 g for 10 min at +4°C. Washing was repeated 10 times with PBS. After washing the homogenate was eluted with 0.02 M citrate buffer pH 3.2 (10 ml of buffer for 1 g of kidney tissue) at 37°C under constant stirring for 2 hours and centrifuged at room temperature at 23 000 g for 10 min. Supernatant was neutralized with NaOH 0.1 N to pH 7 and dialysed to PBS. The protein concentration was determined and the eluate was concentrated by negative pressure in Visking dialysis bags. Concentrates with a protein concentration of about 60 mg/100 ml and 500 mg/100 ml were used for indirect fluorescent studies. Concentrates were analysed using cellulose acetate membrane electrophoresis, immunoelectrophoresis with anti human whole serum, single radial immunodiffusion (19) and double diffusion micromethod (3). Antisera to human IgG, IgA, IgM and β 1C globulin were used in these determinations. The sensitivity of double diffusion technique used for serum immunoglobulins is 0.5 mg/100 ml.

RESULTS

Light microscopy. The histological picture of both cases differed markedly (Figs 1 and 2). In case 1 the changes were slight but distinct. Most glomeruli showed slight to moderate mesangial cell proliferation with increase of PAS and silver positive fibrillar material in mesangial areas (Fig 3). Thickening or splitting of peripheral capillary basement membranes was rarely found. In some glomeruli the urinary space was widened and occasional foetal microglomeruli were seen. Cystic dilatation of proximal tubules was rare. Hyaline casts were seen in some dilated proximal and distal tubules. No interstitial fibrosis nor accumulations of inflammatory cells were present.

In case 2 the pathological changes were



Fig 4 Electron microscopical picture of a glomerulus from case 1. There are hyperplastic mesangial areas. Collections of tortuous basement membrane like material are conspicuous. Epithelial foot processes have disappeared and numerous epithelial microvilli are present. Hypertrophic endothelial cytoplasm is seen

in some capillary loops and bulging of mesangial cytoplasm into a capillary loop is seen. The basement membrane itself is thin. *M* Mesangial area *m* mesangial cytoplasm bulging into the capillary loop *V* epithelial microvilli *E* endothelial cell with abundant cytoplasm *C* capillary lumen $\times 3250$

more severe. Approximately half of the glomeruli showed mesangial reaction as in the previous case. The rest of the glomeruli were in various stages of hyalinization and fibrosis

and showed proliferation of several types of glomerular cells. In several glomeruli cystic dilatation of the urinary space and atrophy of the glomerular tuft was seen (Fig 2). Ex-

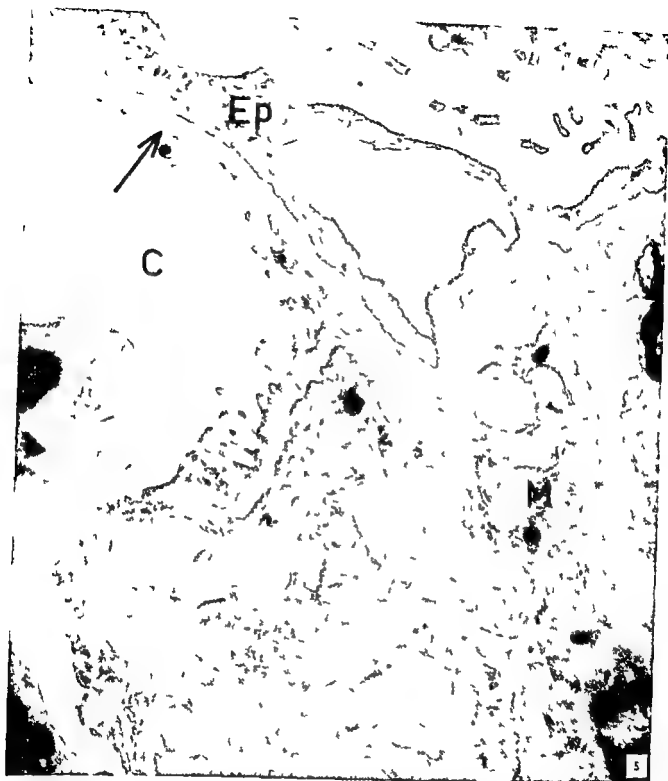


Fig 5 Detail of a capillary mesangial junction. Tortuous material with a density like that of basement membrane is accumulated in mesangial area and it is partly projecting in the capillary space. Foot pro-

cesses have disappeared from the epithelial covering of the basement membrane. M mesangial area; C capillary lumen; Ep epithelial cytoplasm; arrow shows basement membrane. $\times 17,500$.

tensive microcystic dilations of both proximal and distal tubules governed the picture. The dilated tubules contained numerous hyaline casts. Atrophic tubules were dispersed

between the cystic areas. There was prominent diffuse interstitial fibrosis and accumulations of inflammatory cells mainly close to the capsule.

Electron microscopy Three mature looking glomeruli from each case were studied. Fusion of the epithelial foot processes and numerous microvillous projections of the epithelial cells were a common feature in both cases (Fig. 4). As could be predicted from the light microscopy, mesangial areas were widened and showed tortuous matrix with staining characteristics identical with the basement membrane (Fig. 5). No appreciable granular deposits were found. The number of mesangial cells was clearly increased. The basement membrane was thin in general but in some places its borders on both epithelial and endothelial side were slightly uneven and there was some thickening and mottling of the lamina densa. No electron dense deposits were associated with the basement membrane. Occasional obstruction of capillary loops was caused by increase of endothelial cytoplasm containing well formed organelles and numerous microvesicles. To this was added protrusion of mesangial cytoplasm into the capillary lumen (Fig. 4).

Fluorescence microscopy No glomeruli showed fluorescence with any of the antisera used. Positive staining, however, was found in dense casts of tubular lumens and quite frequently also inside the tubular cytoplasm (Figs. 6 and 7). The tubular casts were stained most frequently by anti β 1C-antiserum, almost as frequently with anti IgG serum, less often with anti IgM serum and only occasionally with anti IgA serum. Cytoplasmic staining could be shown with anti β 1C, IgG and IgM sera but not with anti IgA serum.

Elution experiments The wet weight of the tissue to be eluted was 74 g in case 1, 90 g in case 2 and 130 g in control case. Citrate buffer eluate contained 3.6 mg, 2.5 mg and 2.7 mg of protein per 1 g of tissue respectively. Protein concentrations of the eluates for the following analysis were 460 mg/100 ml, 470 mg/100 ml and 540 mg/100 ml respectively. Electrophoresis on cellulose acetate membrane showed protein in the gamma globulin region only in eluates of nephrotic

kidneys. In the eluate of control kidney there was in addition to gammaglobulin a protein in the β region. On double diffusion plates only IgG could be detected in all three eluates. The IgG concentration in case 2 and the control was about 5 mg/100 ml and in case 1 above 2 mg/ml but could not be exactly quantitated. The eluted IgG calculated from these values is approximately 15–35 μ g per gram of kidney tissue and no difference between the amounts of nephrotic kidneys and the control was apparent.

When kidney sections of a normal surgical biopsy were incubated with the eluates in two different concentrations (60 mg/100 ml and concentrations as stated above) and subsequently stained with FITC conjugated antisera, no specific staining of glomerular or tubular components was found.

DISCUSSION

We cannot offer reasonable explanation for differences in the immunofluorescence studies on kidneys of CN children presented by various authors. It is possible that all nephrotic syndromes beginning in early infancy do not belong to the same aetiological group. By reviewing the published results this seems not to be the explanation of some of the contradictory results. All cases of Kouvalainen (12) and those of Hoyer et al. (10) and our were of Finnish extraction suggesting a common genetic origin (22). As far as one can judge from the published reports the clinical course and kidney morphology was very much alike in these cases. It is possible that at the terminal phase of the disease new additional pathogenetic mechanisms can appear with concomitant immunologic injury. Five out of the 6 cases presented by Kouvalainen were autopsy studies and in the kidney obtained at autopsy by Hoyer et al. some immunoglobulin staining in the glomeruli was demonstrated. Our first case represents a morphologically rather early phase of the disease while the second case is an advanced one. The tissue

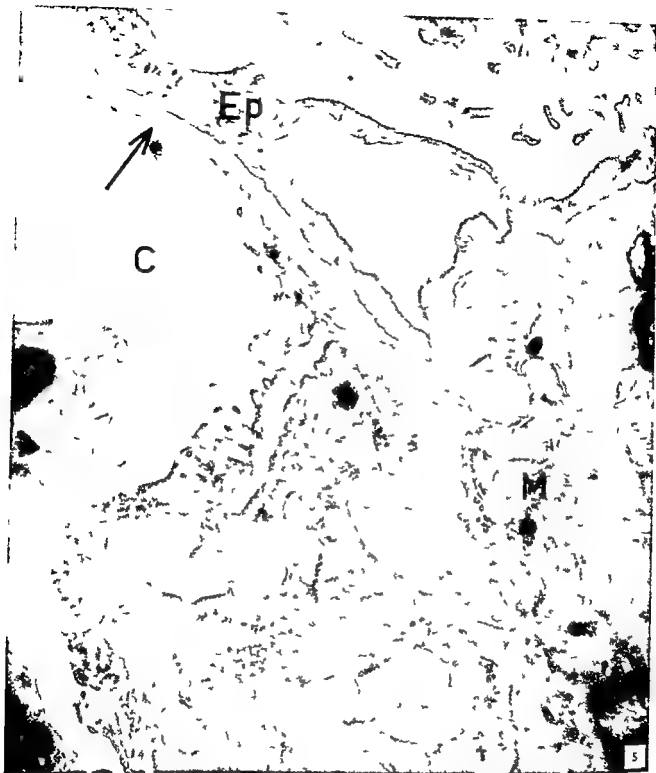


Fig 5 Detail of a capillary mesangial junction. Tortuous material with a density like that of basement membrane is accumulated in mesangial area and it is partly projecting in the capillary space. Foot pro-

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between the cystic areas. There was prominent diffuse interstitial fibrosis and accumulations of inflammatory cells mainly close to the capsule.

however was obtained fresh and may thus be better suited for this type of study than tissue obtained at autopsy

It is generally known that immunofluorescent techniques even to day are capricious. Tissue handling purity, specific antibody titer and labelling of the antiglobulins, optical system and finally interpretation of the staining may affect the results. This is especially true when the amount of antigenic material is present in small quantities. In addition to the usual control procedures we have further tested the monospecificity of our antihuman immunoglobulin antisera and their ability to stain intracellular immunoglobulins and the lack of crossreactivity with gastrointestinal specimens (23).

Electron microscopy gave further support to the absence of immunocomplexes in the glomeruli. We did not see clear nodular deposits on the epithelial side of the basement membrane as seen in poststreptococcal glomerulonephritis (21) or on the endothelial aspect as occurs in lupus erythematosus (7). Thickening of the capillary basal membrane as seen in case 2 was focal and local and did not correspond to the changes in the full blown glomerulonephritides caused by anti-basement membrane antibodies as in Goodpasture's syndrome (5). Enlarged mesangial areas showed increased matrix (basement membrane like material) but no granular electron dense deposits in any appreciable amount as in the anaphylactoid purpura with kidney involvement (25). Close correlation between electron dense deposits and immunoglobulin binding has been observed in these disorders.

In recent studies it has been possible to elute and characterize antibodies from immunocomplexes in kidneys with the antibody coated membrane type of glomerulonephritis (16, 20), lupus nephritis (13) and experimental streptococcal glomerulonephritis (17). Although our immunofluorescent studies appear to make it unlikely that immunocomplexes exist in glomeruli of our patients we wanted to check this with elution experiments. An

additional reason for elution experiments was the unexplained fluorescent staining of the tubular epithelium. This staining combined with the accumulation of lymphoid cells between the tubules led us to suspect the possibility that autoimmune mechanisms might exist as a consequence of the tubular destruction. The fluorescence would thus represent antibodies directed toward autologous tubular antigens. If this were true it could later result in an immunocomplex type of glomerulonephritis as occurs in the experimental allergic glomerulonephritis caused by antitubular antigens (6). The acid eluate contained a minimal amount of antigenic IgG which was also recovered from the control kidney. Lerner et al (16) eluted about 100 μ g of IgG per gram from a glomerulonephritic kidney with Goodpasture's syndrome. Our experiment showed only $1/3$ or less IgG of that value. The indirect immunofluorescent staining with eluted protein did not support our hypothesis of antitubular antibodies. For firm conclusions however more controlled experiments are required to prove or disprove this hypothesis. Unfortunately no material with established immunocomplex disease was available to control the effectivity of the elution experiments.

Our present immunofluorescent studies supported with electron microscopic findings did not disclose any evidence for the presence of immunological mechanisms in the pathogenesis of the congenital nephrotic syndrome in accordance of the earlier results of Hoyer et al (10).

SUMMARY

Two kidneys from patients with congenital nephrotic syndrome obtained at nephrectomy at the age of 3 and 23 months were investigated for possible immunopathological mechanisms for the kidney injury. Immunofluorescent staining with anti IgG, IgM, IgA, and β 2C globulins did not show attachment of immunoglobulins or complement to the

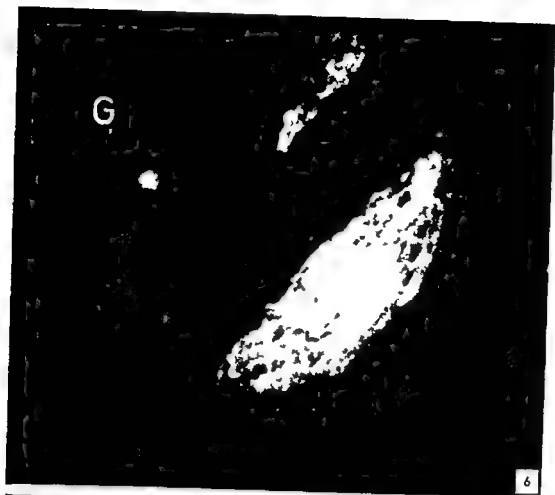


Fig 6 Kidney section of case 2 stained with anti IgG. Casts in the dilated tubules show positive fluorescence as the glomerulus (G) is quite negative $\times 300$

Fig 7 Kidney section of case 2 stained with anti p1C globulin. Faint staining of the epithelium of dilated tubules (T) is visible. Bright intraepithelial staining of a small tubule (t) is evident $\times 300$

however was obtained fresh and may thus be better suited for this type of study than tissue obtained at autopsy

It is generally known that immunofluorescent techniques even to day are capricious. Tissue handling, purity, specific antibody titer and labelling of the antiglobulins, optical system and finally interpretation of the staining may affect the results. This is especially true when the amount of antigenic material is present in small quantities. In addition to the usual control procedures we have further tested the monospecificity of our antihuman immunoglobulin antisera and their ability to stain intracellular immunoglobulins and the lack of crossreactivity with gastrointestinal specimens (23).

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In recent studies it has been possible to elute and characterize antibodies from immunocomplexes in kidneys with the antibody membrane type of glomerulonephritis (16, 20), lupus nephritis (13) and experimental streptococcal glomerulonephritis (17). Although our immunofluorescent studies appear to make it unlikely that immunocomplexes exist in glomeruli of our patients we wanted to check this with elution experiments. An

additional reason for elution experiments was the unexplained fluorescent staining of the tubular epithelium. This staining combined with the accumulation of lymphoid cells between the tubules led us to suspect the possibility that autoimmune mechanisms might exist as a consequence of the tubular destruction. The fluorescence would thus represent antibodies directed toward autologous tubular antigens. If this were true it could later result in an immunocomplex type of glomerulonephritis as occurs in the experimental allergic glomerulonephritis caused by antitubular antigens (6). The acid eluate contained a minimal amount of antigenic IgG which was also recovered from the control kidney. Lerner et al. (16) eluted about 100 μ g of IgG per gram from a glomerulonephritic kidney with Goodpasture's syndrome. Our experiment showed only $1/3$ or less IgG of that value. The indirect immunofluorescent staining with eluted protein did not support our hypothesis of antitubular antibodies. For firm conclusions however more controlled experiments are required to prove or disprove this hypothesis. Unfortunately no material with established immunocomplex disease was available to control the effectivity of the elution experiments.

Our present immunofluorescent studies supported with electron microscopic findings did not disclose any evidence for the presence of immunological mechanisms in the pathogenesis of the congenital nephrotic syndrome in accordance of the earlier results of Hoyer et al. (10).

SUMMARY

Two kidneys from patients with congenital nephrotic syndrome obtained at nephrectomy at the age of 3 and 23 months were investigated for possible immunopathological mechanisms for the kidney injury. Immunofluorescent staining with anti IgG, IgM, IgA and β 2C globulins did not show attachment of immunoglobulins or complement to the

glomeruli. Staining was found in some tubular casts and occasionally in tubular epithelium as well. Electron microscopic studies of the glomeruli corroborated the idea that immunocomplexes are not conglomerated in the glomeruli because of the absence of typical deposits which are usually found in association with positive immunofluorescent staining in various glomerulonephritides.

Kidneys were also eluted with acidic buffer in order to remove antibodies from immunocomplexes. The eluates were analyzed for their immunoglobulin content and used for indirect immunofluorescent staining. The content of IgG of the kidney eluates was low and no difference of eluted IgG between the nephrotic kidneys and the control was apparent. No binding of immunoglobulin with structures of normal kidney could be seen in the indirect immunofluorescent study.

Our results corroborate the previous results (10) that immunopathogenesis is not essential in the congenital nephrotic syndrome.

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HYPOSENSITIZATION IN HOUSE DUST ALLERGY ASTHMA

A Double blind Controlled Study with Evaluation of the Effect on Bronchial Sensitivity to House Dust

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A placebo controlled study of the effect of hyposensitization was performed in 93 children shown to suffer from asthma caused by bronchial allergy to house dust. The allergy diagnosis was substantiated by bronchial provocation tests (BPT) and the effect of the injection treatment was assessed by estimation of the degree of bronchial sensitivity to house dust before and after treatment (2). The specific hyposensitization was found to be superior to placebo injections as concerned improvement of allergic responsiveness of the bronchi. The data relevant to the study of the effect on bronchial sensitivity and subjective improvement are presented in this paper.

MATERIAL AND METHODS

The patients were selected among children 2-14 years old who had been referred for bronchial asthma. Only those who had such severe asthma that active treatment with hyposensitization seemed justifiable were included in the study. Additional requirements were that the patients reacted with bronchial obstruction to bronchial provocative tests with both of the two house dust extracts used in this study and that they had not received hyposensitization with house dust extracts prior to the study.

The same allergen extracts were used both for skin testing, bronchial provocative tests and hyposensitization. Two house dust extracts were purchased from Nyegaard et Co. Oslo (house dust A) and from Allergologisk Laboratorium Copenhagen (House dust B) respectively. Saline (0.9 per cent) containing 0.5 per cent phenol was used as placebo and a brown

sugar stain was added to make the placebo look similar to the house dust extracts. Both extracts and placebo were similarly bottled in randomly coded vials by Nyegaard et Co. and the top concentration was indicated as 1:10. The code was broken after the final evaluation of the effect of treatment.

The diagnostic procedures used for the selection of patients were as described in previous papers (1). The bronchial provocation tests were initiated at low concentrations of the allergen extract and the concentration was then gradually increased. The minimal dose needed to elicit significant bronchial obstruction was recorded as the particular bronchial sensitivity threshold dose (2).

The hyposensitization treatment was undertaken by the local physician who had referred the patient. Using this approach it was assured that local reactions elicited by the extract injections could not be registered by the persons who finally evaluated the treatment. The injection treatment was started with the extract or placebo diluted with saline containing 0.5 per cent phenol to a concentration of 1:1 million. Injections were given 1-2 times weekly until 0.5-0.9 ml of the top concentration was reached. If reactions to the injection occurred the physician was instructed to reduce the dose and continue with the highest tolerated dose for maintenance. The maintenance dose was given with 2 to 4 week intervals and this was continued for 2 1/3 years until the re-evaluation of the bronchial sensitivity.

The effect of the treatment was evaluated by means of repeated bronchial provocative tests with estimation of the specific bronchial sensitivity threshold to the house dust extracts. When no bronchial reaction could be provoked by the inhalation of 1 ml of the top concentration (1:10³) of the extract the improvement of bronchial tolerance was registered as 100 per cent. Partial improvement was recorded accordingly. The actual increase of the bronchial threshold dose was expressed as the percentage of the increment between the starting threshold dose and

Table 1 Data for patients omitted from the study

Patient code	Reason for omission	Treatment group	Improvement	
			Subjective	Bronchial
9	Too much asthma	A	O Corticosteroid dependent	Not assessed
13	Stopped because of local and eczematous reactions after 10 months treatment	A	Much improved	100%
27	Too much asthma	A	No change	0
49	Switched to known genuine extract	A	Symptom free	100
74	BPT not performed after treatment	A	Much improved	Not assessed
14	Stopped because physician does not believe in the treatment	B	Some improvement	0
41	Injectons too irregularly given	B	Much improved	75
61	Too much asthma	B	Corticosteroid dependent	Not assessed
33	Switched to known genuine extract	C	Much improved	100%
51	BPT not performed after treatment	C	Much improved	Not assessed
66	Switched to known genuine extract	C	Much improved	Not assessed
94	Injectons not given as instructed	C	Much improved	0
96	Stopped because physician does not believe in the treatment	C	No change	0

1 ml of the top concentration. For this approximation this increment was plotted on a linear scale each 1:10 dilution represented by identical values. Care was taken to perform the bronchial provocation tests under conditions comparable to those of the usual ones (*).

Data were collected also for assessment of the general clinical condition before, during and after the injection treatment. These comprised symptom diaries, records of medication used, days lost from school and the parents' impression of the degree of improvement during the house dust season (October-March). In the present report weight is given only to the effect on the bronchial sensitivity to house dust, but this effect will be compared with the subjective impression of the parents.

RESULTS

Thirteen of the 93 patients originally admitted to the study had to be omitted for reasons summarized in Table 1. Five of the omitted patients belonged to the group "House dust A", 3 to the group "House dust B" and 5 to the group receiving placebo injections. The bronchial sensitivity and subjective improvement were assessed in 8 of the 13 patients omitted (Table 1).

The study was completed for 80 patients. House dust A had been given to 31, house dust B to 21 and placebo to 28 patients respectively (Fig. 1). In the house dust A and house dust B treated groups complete bronchial tol-

erance to the house dust provocations had been achieved in 21 and 14 patients respectively compared with only 7 in the placebo group. When those with marked improvement of bronchial tolerance (more than 75 per cent of maximum possible improvement) were included the figures were 26/31, 19/21 and 9/28 respectively—showing that both house dust extracts were significantly superior to the placebo ($p < 0.01$).

Changes of bronchial sensitivity before and after the injection treatment were compared with the clinical improvement as subjectively judged by the parents (Fig. 2). Most of the house dust treated patients showed marked improvement and most of the placebo-treated patients did not with respect to both parameters but full correlation was not observed. By subjective measures 2 patients were classified as worse or unchanged although full tolerance to the house dust bronchial provocation had been achieved and 6 patients were classified as much better although the bronchial sensitivity to house dust appeared to be unchanged or even worse.

DISCUSSION

Bronchial allergy is a frequent cause of asthma (1, 2). Several allergens precipitate attacks of

school or work reduction of symptomatic medication and subjective well being are as much indicators of changes of the patients attitude to the disease and effectiveness of simultaneous therapy as they are of benefits of the injection treatment on the bronchial reactivity as such

Even when placebo-controlled studies are performed errors may arise leading to false conclusions. Assessment of therapeutic effect is not possible if the diagnosis is inconclusive or incorrect. Other critical points to be considered are selection of patients, diagnostic criteria, quality of allergen extract and placebo, optimal use of the therapeutic measure to be studied and parameters for the evaluation of the effect (12, 13). Several double blind controlled studies suggest that specific hyposensitization is superior to placebo injections in the treatment of allergic diseases (4, 6, 7, 11, 12, 16). However, a few negative reports have appeared. Fontana et al. (3) conducted a double-blind study in children with ragweed hay fever. The parents kept a symptom record which was finally scored for the presence or absence of symptoms whereas the severity and duration of symptoms were not noted. There was no difference between the treated group and the placebo group in this study. Critical objections are that the treatment apparently was too short and the type of evaluation used did not detect any amelioration less than 100 per cent. One report presented by the Research Committee of the British Tuberculosis Association (BTA) suggested that hyposensitization with house dust was ineffective in bronchial asthma (15). However, this study did not fulfill the demands of a scientific approach to treatment evaluation (5). The diagnosis of bronchial allergy to house dust was made by history and skin tests only. The specific allergy diagnosis may be subject to a 20-50 per cent methodological error when based on such criteria only (1, 2). Albeit previous studies have repeatedly shown that hyposensitization should be carried out with a maximum tolerated dose and for a rather prolonged time (4, 6, 7, 11, 12, 16) the treatment in the

BTA study consisted of a rather short course of injections following either a routine non individualized set up or a course which was brought to an end if the patient showed untoward reactions. The evaluation was based on the patients subjective impression. Obviously this sort of study is of restricted value.

The results of several other controlled studies suggest that hyposensitization with selected allergens is effective and specific provided the doses are large enough and that the treatment is prolonged (4, 6, 7, 11, 12, 13, 14). This has also been substantiated by experimental studies with sensitized animals and sensitized cells in cell suspensions. The latter studies have shown that hyposensitization has positive effects also at the cellular level although the basic mechanisms at work are not clarified (8, 9, 10, 14, 16, 17, 19).

The lack of controlled information about the effect of hyposensitization on the bronchial hypersensitivity to the particular allergen has been a kind of missing link in the chain of evidence. The results of the present study appear to consolidate this chain.

SUMMARY

A double blind controlled study of the effect of house dust hyposensitization was conducted in 93 patients allergic to house dust. The allergy diagnosis was established by bronchial provocation tests and the degree of bronchial sensitivity to house dust extracts (bronchial threshold dose for allergen) was assessed before and after 2½-3 years injection treatment.

The specific hyposensitization with two different house dust extracts was superior to placebo injections.

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PROTHROMBIN IN NEWBORNS AND DURING THE FIRST YEAR OF LIFE

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The hypoprothrombinemia of the newborn was first demonstrated by Brunkhous *et al.* in 1937 by use of their two-stage prothrombin assay (5). Waddell and coworkers (41) were the first to associate the hypoprothrombinemia expressed by a prolonged "prothrombin time" (34) with vitamin K deficiency. Several investigators (7, 29, 30, 35) reported that prothrombin decreased during the first few days of life and then increased to normal values within a week (35) or within a year (5, 6, 30) depending on the method used for the assays. It has been postulated that the postnatal decrease of prothrombin and related coagulation factors might be explained by a quick reduction of the transferred maternal factors combined with a slow synthesis of coagulation factors in the infant liver (16). Because of the potential bleeding risk in this age period, vitamin K prophylaxis was introduced and a safe dosage established (1, 6, 10, 36).

Studies of prothrombin and other coagulation factors in the newborn have resulted in some paradoxical findings. Thus the one-stage prothrombin time according to Quick (34) gives normal values in newborns in spite of definitely subnormal activities of factors VII, X and prothrombin (factor II) assayed by specific methods. In adults the Quick test registers deficiencies of these factors. Several ex-

molecular dif-

ferences between infant and adult prothrombin (30, 36) and fibrinogen (20, 42) or possibly an excess of factor V (11) in the newborn. It is evident that testing techniques used must always be taken into account when comparing coagulation assay results especially in this age group (1).

In most Swedish hospitals "prothrombin index" according to Quick, Lehmann (23, 34) and the prothrombin proconvertin assay according to Owren & Aas (31) have been replaced by thrombotest according to Owren (32) as the latter test is performed with a ready-made freeze-dried reagent and is well standardized. The Quick-Lehmann test registers deficiencies of factors II, V, VII, X as well as fibrinogen (factor I) but with varying sensitivity. The prothrombin proconvertin and the thrombotest reagents contain bovine fibrinogen and factor V and the methods register deficiencies of vitamin K-dependent coagulation factors, i.e. factors II, VII and X and the thrombotest to a certain extent also factor IX.

The present investigation was undertaken to study a new specific two-stage assay of prothrombin (3, 28) in newborns. Further, the correlation between thrombotest activity and prothrombin concentration was studied as well as prothrombin and proconvertin during the first year of life. The study was especially concerned with cases of decreased prothrombin

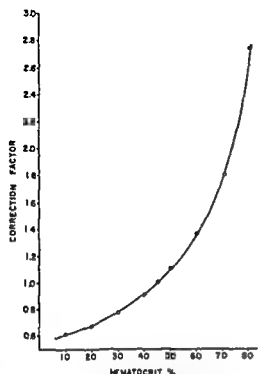


Fig 1 Correction for hematocrit was made by a correction factor calculated according to the expression $F = (100 - Hn)/(100 - H)$ Hn normal mean value for hematocrit (45% used here) H hematocrit of sample Correction factor (F) was plotted against hematocrit (H)

content, aiming at establishing a borderline value beneath which treatment with additional vitamin K or plasma should be given to prevent bleeding

METHODS

Thrombotest was performed by addition of 0.05 ml capillary blood mixed with 0.05 ml citrated saline to 0.25 ml of reconstituted warmed reagent (37°C) and measuring the clotting time (19)

Table 1 Prothrombin determinations (28) in mixtures of citrated plasma and washed red cells

Hematocrit of mixture (%)	Correction factor for hematocrit	Obtained prothrombin value	Corrected prothrombin value
20	0.68	146	102
30	0.78	128	102
40	0.91	108	100
45	1.00	100	100
50	1.10	92	101
60	1.37	72	99
70	1.80	54	98
80	2.75	36	99

Prothrombin was determined with a two-stage assay using a freeze-dried reagent of intrinsic coagulation factors (28). In this method 0.025 ml capillary blood diluted 1:3 with citrated saline is added to 1 ml of reconstituted warmed (37°C) reagent. After a certain incubation time 0.2 ml of this mixture is added to 0.2 ml of warmed (37°C) fibrinogen solution and the clotting time determined by aid of a coagulometer (Depex de Bilt Holland).

Prothrombin and proconvertin (PP) was determined in capillary blood according to Sundblad's modification of Owren's method (31-38). In this modified method capillary blood is diluted with buffered citrated saline. The blood is centrifuged and 0.2 ml of the supernatant is added to 0.4 ml of reagents. The mixture is recalcified and the clotting time determined.

Hematocrit was determined in capillary blood with heparinized microhematocrit tubes (inner diameter 0.5-0.6 mm length 75 mm). The tubes were centrifuged for 3 min at 11,000 rpm in a microcapillary centrifuge (International Equipment Company Boston USA).

Correction for the hematocrit of the blood sample was performed by aid of the curve shown in Fig 1. The validity of this correction was tested on mixtures of citrated plasma and washed red blood cells (Table 1).

MATERIAL

The clinical material included 100 infants aged 0-12 days from the Department of Pediatrics, Karolinska sjukhuset, Stockholm, hospitalized under various diagnoses: hyperbilirubinemia 20, asphyxia 25, respiratory distress syndrome (RDS) 11, immaturity 10, dysmaturity 4, infections 7, VOG 5, other diagnoses 1. Moreover 61 healthy full-term newborns aged 0-12 hours from the Department of Obstetrics at the Karolinska sjukhuset, Stockholm, were investigated, as well as 98 healthy children aged 0-12 months from nursery homes in Gothenburg. All newborns were given 1 mg vitamin K₁ (Konakion®) intramuscularly immediately after birth. In some asphyctic newborns vitamin K₁ was given somewhat later when other vitally indicated treatment had been initiated. No vitamin K₁ was given to the mothers.

The investigation was with short interruptions continued over a period of one year. Samples were taken only on weekdays during day time.

RESULTS

Correlation between prothrombin and thrombotest values

In Fig 2 is shown the correlation between prothrombin and thrombotest values for the entire clinical material with and without correction for hematocrit. As can be seen there is a

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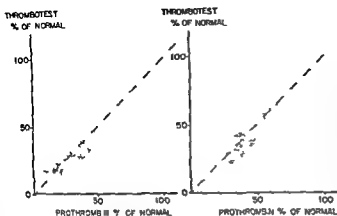


Fig 2 Prothrombin and thrombotest values of the clinical material from Karolinska Sjukhuset I values not corrected for hematocrit II values corrected for hematocrit. Fewer corrected values are shown, because hematocrit was not determined on all occasions

dency for prothrombin values to be somewhat higher than thrombotest values

To investigate whether the slight discrepancy between thrombotest and prothrombin in the newborns could be explained by the presence of an inhibitor as has been shown for dicumarol treated adults (18) plasma samples from 3 newborns and mixed normal adult plasma were analyzed with thrombotest according to Hemker (18). The results are shown in Fig 3. As can be seen there was no evidence for the presence of an inhibitor.

The correlation between prothrombin and thrombotest values is also illustrated in Figs 4, 5, 6 and 7 in which data from individual infants during the course of treatment with vitamin K and plasma are shown. Effect of treatment with plasma and vitamin K was equally well registered with both methods.

Prothrombin and thrombotest in normal full term newborns

Prothrombin and thrombotest values from 61 healthy full term newborns aged 0-24 hours are presented in Fig 1. As can be seen the distribution of prothrombin and thrombotest values was slightly skewed. The logarithms of the corrected values were normally distributed according to the normal equivalent deviate method (NED) (26). The mean value and mean ± 2 SD calculated from the logarithmic

values was for prothrombin 47% and 26-85 for thrombotest 45° and 27-74°. No values were found below 20° in our normal material.

Prothrombin and prothrombin proconvertin during the first year of life

Prothrombin and prothrombin proconvertin (P/P) values from 98 healthy children 0-12

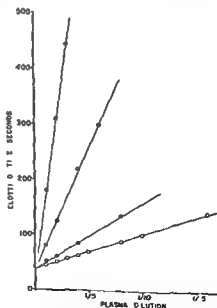


Fig 3 Plasma in different dilutions from neonates and adults was added to thrombotest reagent and the clotting time determined. Clotting time was plotted against dilution ●●● individual neonates ○ mixed plasma from 20 normal adults

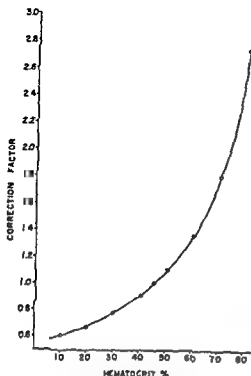


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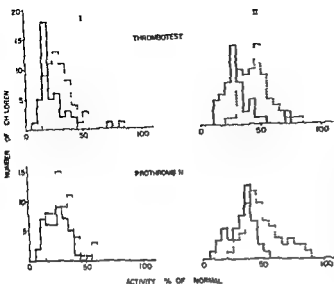


Fig 8 Prothrombin and thrombotest values from 45 hospitalized neonates, aged 0-24 hours, and from 61 healthy full term neonates aged 0-24 hours (with dotted lines) I values not corrected for hematocrit II values corrected for hematocrit

ence was significant ($p < 0.01$) only for prothrombin values. As the hematocrit for the hospitalized neonates was significantly lower ($p < 0.001$) than the hematocrit for the healthy infants, correction for hematocrit increased the difference between the groups.

In the hospitalized neonates there was no significant correlation between birth weight and prothrombin or thrombotest values, nor any sex difference. Correlation between gestational age and prothrombin values was significant ($p < 0.01$) but there was no correlation between gestational age and thrombotest values.

15 of the hospitalized neonates aged 0-24 hours (Table 2) and 5 of the infants aged 1-21 days had a corrected prothrombin or thrombotest value of 20% or lower. This low number could probably be explained by the intense treatment. Some infants were treated with vitamin K and/or plasma even before samples could be taken for the combined investigation of prothrombin and thrombotest.

In our clinical material there were only two infants with manifest bleeding symptoms. One was a boy aged 1 day with respiratory distress syndrome. He had a prothrombin value of 18% and a thrombotest value of 22%. The

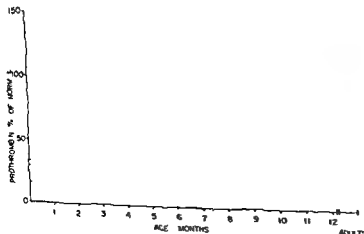


Fig 9 Prothrombin values not corrected for hematocrit, for healthy children during the first year of life.

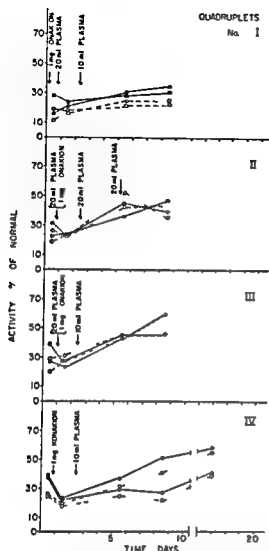


Fig 4 Quadruplets not hormon induced Gestational age 30 weeks I Immature child Birth weight 1260 g II Slightly asphyctic child Birth weight 1190 g III Slightly asphyctic child Birth weight 990 g IV Child with moderate respiratory distress syndrome Birth weight 1210 g

○ prothrombin values not corrected for hematocrit
 ● prothrombin values corrected for hematocrit
 □ thrombotest values not corrected for hematocrit
 ■ thrombotest values corrected for hematocrit

months old, are shown in Figs 9 and 10. These values were not corrected for hematocrit. As can be seen, prothrombin values reach the adult normal range at the age of 6–12 months and prothrombin proconvertin activity at the age of about 3 months.

Prothrombin and thrombotest in hospitalized newborns

Data from the hospitalized neonates aged 0–24 hours, 45 in all, are summarized in Fig 8

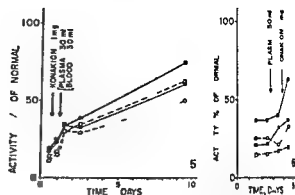


Fig 5 Child of diabetic mother Gestational age 37 weeks Delivery with caesarean section Birth weight 1950 g Moderate respiratory distress syndrome. ○ prothrombin values not corrected for hematocrit ● prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit

Fig 6 Small for date dysmature child Gestational age 41 weeks Birth weight 2430 g ○ prothrombin values not corrected for hematocrit ● prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit

The values from the healthy full term neonates are shown for comparison. Using values corrected for hematocrit, the mean ± 2 SD for the hospitalized neonates was for prothrombin $30\% \pm 27\%$ and for thrombotest $31\% \pm 21\%$. The hospitalized neonates had significantly lower corrected prothrombin and thrombotest values than the normal full term infants ($p < 0.001$). For uncorrected values the differ-

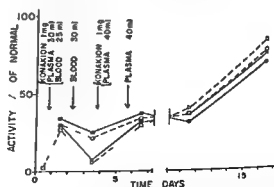


Fig 7 Child of mother with toxemia Gestational age 33 weeks Severe respiratory distress syndrome ○ prothrombin values not corrected for hematocrit ● prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit

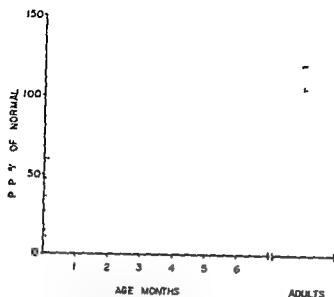


Fig 10 Prothrombin-proconvertin (P-P) values not corrected for hematocrit for children during the first year of life

other infant was a girl, aged 3 days with transposition of the great vessels. She had a prothrombin value of 15% and a thrombotest value of 6% (all values corrected for hematocrit). Both infants were severely asphyctic.

DISCUSSION

In the present investigation a close correlation was found between prothrombin concentration

specifically determined, and thrombotest activity in the newborn child. Thrombotest values, however, tend to be slightly lower than prothrombin values. In the dicumarol-treated adult patients thrombotest gives lower values than specific tests for separate coagulation factors including prothrombin (18). It has been shown that the presence of an inhibitor (18) influences thrombotest in these cases. In the newborn no such inhibitor could be demonstrated. As thrombotest registers deficiencies of all the factors of the prothrombin complex (factors II, VII, IX and X) and is most sensitive to changes of factors VII and X (32), it seems probable that in many cases factor VII or X is more depressed than prothrombin itself, resulting in lower thrombotest than prothrombin values. Other investigators, using one- and two-stage assays of prothrombin, have demonstrated somewhat higher prothrombin than factor VII values in the newborn (24-39). According to Fresh et al (11) the relation between prothrombin and factor VII in the newborn child depends on the amount of vitamin K given and how early the treatment is started. In our material too few infants were followed during treatment with vitamin K in varying doses to allow any conclusions about relations of prothrombin and factor VII values ex-

Table 2 Infants aged 0-24 hours with prothrombin or thrombotest values (corrected for hematocrit) of 20% or lower

Sex	Patient	Prothrombin		Thrombotest		Diagnosis
		Uncorr. value	Corr. value	Uncorr. value	Corr. value	
♀	II	13	14	5	—	Asphyxia immaturity
♀	SI	10	16	16	25	Asphyxia immaturity twin
♀	SII	11	9	17	14	Asphyxia immaturity twin
♀	C	12	21	8	14	Asphyxia severe
♀	L	21	25	17	20	Asphyxia
♀	M	8	10	12	16	Asphyxia immaturity
♀	S	17	25	12	17	Asphyxia
♀	H	15	18	12	14	RDS
♀	K	12	17	13	19	RDS
♀	L	8	12	17	25	RDS
♀	B	16	18	20	22	RDS intrapulmonary and intracerebral hemorrhages
♀	SI	11	17	11	29	Immaturity quadruplet
♀	C	24	29	15	18	Neonatal pneumonia
♀	A	17	19	20	22	Hypoalbuminemia
♀	L	27	36	14	19	Diabetic mother

pressed by thrombotest at different levels of vitamin K dosage. However our results do not contradict the findings of van Meer (25) that during high speed synthesis of prothrombin and related factors factor VII values are higher than prothrombin values and at low speed synthesis factor VII values are lower than prothrombin values.

Our finding that prothrombin reaches normal adult level at the end of the first year of life is in accordance with several other investigators (5, 6, 30). Prothrombin proconvertin values reach the normal adult level at the age of about 3 months as does factor VII according to Dyggve (9).

In contrast to several other investigators (13, 22, 40) we found it reasonable to correct prothrombin and thrombotest values for hematocrit as prothrombin and related factors are distributed in plasma and the hematocrit shows great variability in this period of age. The distribution of prothrombin values thus corrected showed good agreement with the values obtained by Nihlen & Ganrot with an immunological assay in plasma samples from neonates (12, 27).

The lower limit, calculated from the mean logarithmic value -2 S.D. of our normal full term neonates was for prothrombin 26 and for thrombotest 27 (corrected values). In our normal material no values were found below 20°.

Bleeding complications were rare in our material probably because of intense treatment with vitamin K and plasma. No manifest bleeding was observed in any patient with prothrombin above 20° and thrombotest above 22 (corrected values). In the bleeding cases influence of other factors than the prothrombin concentration might be of decisive perhaps additional importance.

Among the cases with decreased prothrombin and/or thrombotest values children suffering from hypoxia prevailed. Three of these children were given vitamin K 2 hours after instead of immediately after birth but this could hardly explain the finding. A depressing

effect of hypoxia on prothrombin and other coagulation factors has been reported and discussed by many authors (1, 2, 8, 14, 15, 17, 20, 21, 37 and others). In recent animal experiments it has been shown (15) that prolonged periods of hypoxia in the newborn dog gives depression of factors II, V, VII and X.

The three test methods used in our investigation are all of value for clinical use. As the reagents for the prothrombin proconvertin method have to be prepared by each laboratory and stored at -20°C our interest has been focused on the prothrombin and thrombotest assays for which freeze-dried ready made reagents are supplied. The prothrombin assay is carried out in two steps and is consequently somewhat more cumbersome technically than thrombotest which is a one stage method and easy to perform. On the other hand the prothrombin assay might be used for the whole range of prothrombin concentrations while thrombotest is less reliable in the range 50–100% (33).

SUMMARY

Prothrombin concentration determined specifically with a new two-stage method was compared with thrombotest activity in samples from infants aged 0–21 days. There was a significant correlation between the two methods.

Effect of treatment with vitamin K and plasma was registered with both methods.

As the distribution of prothrombin and thrombotest values from normal full term neonates was slightly skewed mean value and S.D. was calculated from the logarithmic values. The normal range expressed as mean ± 2 S.D. was for prothrombin 26–85° and for thrombotest 27–74° (values corrected for hematocrit).

No bleeding was observed in any patient with prothrombin above 20° and thrombotest above 22° (corrected values). Most low values in newborns were found in children suffering from hypoxia.

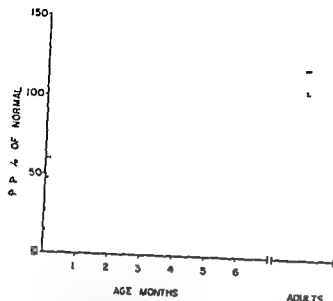


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ACKNOWLEDGEMENTS

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CIRCULATORY ADAPTATION IN THE THERMOREGULATION OF FULLTERM AND PREMATURE NEWBORN INFANTS

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Important contributions to our knowledge of thermoregulation in the newborn have been made during recent years, both as regards the control of thermogenesis (2, 8, 9) and the control of heat loss by regulation of cutaneous circulation (2). The meticulous work by Bruck and co-workers is outstanding in this respect, and the reader is referred to the review by Bruck (2) for a full discussion of this subject. In these latter studies skin blood flow was estimated by measurements of the thermal conductivity coefficient according to Hensel & Bender (7).

Since more quantitative methods to determine the rate of circulation in the extremities of newborns have become available, important differences to the adult have been established (1, 3, 4, 5, 6). Such data provide a useful background when circulatory adjustments in thermoregulation are considered. Besides giving information on the rate of blood flow in quantitative terms, the plethysmographic method may have certain advantages as the local effect on circulation may be studied separate from reflexogenic effects of induced temperature changes on remote parts of the body surface.

The present report is based on observations obtained from a large number of infants carried out with the aim of examining at first hand the capacity of the newborn to control peripheral circulation in relation to the surrounding temperature.

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MATERIAL AND METHODS

The majority of our subjects were fullterm infants which upon repeated examination by a paediatrician were considered normal and healthy. They were taken from the wards to the laboratory preferably between their regular feeding hours. The laboratory was thermostatically maintained at 23°C. A suitable plethysmograph was fitted to the right foot and calf after which the infant was put into an Isolette incubator. The blood flow recording was performed as previously described (3). After a period of approximately 30 min the infants had mostly fallen asleep and the rate of limb blood flow had reached a steady level. Series of blood flow determinations were then performed at short intervals as the temperature in the incubator (ambient temperature) or the temperature of the water within the plethysmograph (local temperature) or both these variables were gradually lowered.

Temperature of the incubator

The temperature at the start of the experiment was kept at different levels within the range from 30°C to 35°C. This was done in order to determine basal blood flow at different temperature levels (see Table 1). A gradual lowering of the temperature was achieved by turning off the heating filling the ice box with ice and letting the side holes of the incubator hood remain open. A thermocouple was suspended just over the chest of the infant and the temperature within the incubator was continuously recorded on an electrothermometer. We preferred to let the exposure of the child to a colder environment take some time rather than to create a more abrupt change since the final adaptation to a lower temperature was considered as the most important object of our study. Therefore the fall of temperature was allowed to proceed at an approximate rate of 0.5°C per min.

Temperature of the plethysmograph

The original method to maintain the temperature of the water in the plethysmograph constant (3) was altered to a system of water circulating through the

Table 1 *Resting limb blood flow at different levels of ambient temperature and local temperature (mean values from 131 fullterm healthy newborns)*

	Subgroup	No of infants	Ambient temp (°C)	Local temp (°C)	Limb blood flow (ml/min/100 ml)	S D
More than 12 hrs of age	A	57	30	34	7.3	±2.7
	B	20	33-35	34	8.2	±2.2
	C	15	30	40	8.9	±2.5
Less than 12 hrs of age	D	26	30	34	5.4	±2.5
	E	13	30	40	7.3	±2.6

plethysmograph from a reservoir outside the incubator. The inlet of water from the reservoir and the outlet of water from the plethysmograph was adjusted by screw clamps, so that the volume of water in the plethysmograph remained constant. During the experiment the temperature of the water in the plethysmograph could thus be kept constant or it could be altered by this simple device. This circulation of water through the plethysmograph was interrupted during the actual measurements of blood flow. A thermocouple inserted into the plethysmograph provided a continuous recording of the temperature of the water in the plethysmograph. In the majority of children rectal temperature was also continuously recorded by way of a rectal thermocouple.

General experimental conditions

All measurements were carried out on infants during natural sleep. The exposure to a cold environment had a marked arousal effect. Each experiment was carried on until the infant became restless or woke up. Changes in wakefulness and motor activities that sooner or later became prominent, were recorded.

The following series of experiments are included in this report.

1 The resting blood flow was determined in 131 fullterm newborn infants at different combinations of ambient and local temperatures within the assumed "neutral zone". This series is divided into five different subgroups, based on differences in temperature combinations and in postnatal age (see Table 1).

The circulatory response to a cold stimulus induced by a lowering of ambient temperature, local temperature or both was studied in additional 46 infants. These studies were divided into four main subgroups (see Table 2).

In 13 fullterm infants, ambient temperature was lowered whereas local temperature was maintained constant. Series A.

3 In 8 fullterm infants, both ambient and local temperature was lowered. Series B.

4 In 17 fullterm infants, local temperature was lowered whereas ambient temperature was maintained constant. In Series C, ambient temperature was close to the lower limit, and in Series C, close to the upper limit of the assumed neutral zone.

5 Fourteen exposures to a lowering of ambient

temperature were performed on 8 premature or low birthweight infants of different gestational and postnatal age. Series D and D₁.

There were no complications whatsoever while performing these studies.

RESULTS

1 Resting blood flow in the foot and calf at different levels of ambient temperature and local temperature

Data obtained on measurements on 131 healthy fullterm newborns are summarized in Table 1. When flow was measured at 30°C ambient temperature and 34°C local temperature (group A) the mean value for this group was 7.3 ml/min/100 ml. This is of the same order of magnitude that was found in previous series (3, 5). Either an increase of ambient temperature (group B) or of local temperature (group C) raised the rate of local blood flow some 15 to 20 per cent. The one conclusion that may be drawn is that both ambient temperature and local temperature influence the rate of peripheral blood flow to some extent and that this fact has to be carefully considered in studies on peripheral circulation.

Group D and E in Table 1 refer to measurements during the first 12 hours of life. These observations show that under identical temperature conditions the rate of blood flow is lower at this early stage but also that an increase of local temperature may still provoke a vasodilatation. This restriction of peripheral circulation during the first hours of life also limits the maximal blood flow capacity at reactive hyperaemia (6).

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Temperature of the incubator

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Temperature of the plethysmograph

The original method to maintain the temperature of the water in the plethysmograph constant (3) was altered to a system of water circulating through the

Table 3 The circulatory response to lowered ambient temperature on 8 premature healthy infants (local temperature not maintained constant)

No	Birth weight (g)	Gestat period (weeks)	Post natal age (days)	Temp of incubat. (°C)	Temp of pleth. (°C)	High temp of blood flow (ml/min/100 ml)	Temp of incubat (°C)	Low temp of blood flow (ml/min/100 ml)	Ratio	Blood flow at low temp Blood flow at high temp
1	1600	30	6	32.5	34	7.5	29	2.4	0.35	
			17	32.5	34	7.5	28	3.5	0.45	
			36	32	34	16.7	28	5.8	0.35	
2	1450	30	42	32	34	13.7	30	10.7	0.80	
			4	32	34	7.0	29.5	4.3	0.60	
3	1930	32	22	32	34	16.9	29	3.5	0.70	
			3	32.5	34	5.0	29	3.5	0.20	
4	1500	33	7	32.5	34	11.7	29	2.2	0.35	
5	1810	35	15	33	36.5	17.4	28	6.3	0.75	
			5	32.5	34	4.5	27.5	3.4	0.45	
6	1830	37	21	32.5	34	18.7	27.5	8.8	0.45	
			9	32	34	11.6	28.5	5.4	0.60	
7	1870	36	23	32	34	12.4	30	7.3	0.30	
			21	32	34	23.0	26.5	6.8		

30°C which is at the lower limit of the assumed "neutral zone". The thermal stimulus was a progressive lowering of the temperature in the plethysmograph from the initial level of 35°C to around 26°C. In this series there was a rather pronounced vasoconstrictor response from 6.4 to 2.6 ml/min/100 ml of tissue which is a highly significant difference. As the stimulus of a lowered temperature was limited to the area from which flow determinations were made, there are two possible mechanisms by which a vasoconstrictor response may be obtained. In the first hand, a direct effect on the cutaneous blood vessels has to be considered. And secondly, there is the possibility that the reduction of blood flow is even under these experimental conditions at least partly due to a true thermoreflex over hypothalamic thermoregulating nervous structures. That the latter mechanism is quantitatively the more important is shown by the findings in Series C₂. Here the local cold stimulus was much stronger as the temperature of the water in the plethysmograph was gradually lowered from 35°C to 15–22°C. But despite this stronger local cold stimulus the reductions in blood flow in these infants were less prominent (from 6.6 to 5.0 ml/min/100 ml of tissue). The difference in experimental conditions in Series C₁ and C that

explains this increased resistance to a local cold stimulus is the higher general ambient temperature that was applied to the infants in Series C. An ambient temperature of 35°C (instead of 30°C) over the major part of the body surface obviously protects the infant from a more prominent reflex vasoconstriction even at a marked drop of local temperature (13–20°C). On the other hand, if ambient temperature is kept close to the lower limit of the "neutral" zone (30°C) a relatively much smaller change of local temperature on the foot and calf (8–10°C) may suffice to start a more prominent reflex vasoconstriction.

5 Circulatory response in premature infants to lowered ambient temperature, local temperature not maintained constant

This series was included to find out whether or not active thermoregulation is developed at premature birth. In a more detailed study on premature infants (1) we have noticed that premature infants generally have a very high rate of limb circulation under resting conditions and also that the maximal blood flow capacity at reactive hyperaemia is correspondingly higher—both studies indicating a high vascularity. We have also found that when prema-

Table 2 The circulatory response to lowering of ambient temperature, local temperature, or both in four different experimental series. Temperatures and mean values of local blood flow before and after exposures and the probability of the blood flow differences are given. \times marks when local temperature was allowed to cool with the ambient temperature

Series	Subjects	n	Age	Temperature °C				Blood flow (mean ± S E ml/min/ 100 ml tissue)		Probability of the flow difference
				before		after		before	after	
				ambient	local	ambient	local			
A	Normal healthy fullterms	13	6 hrs- 5 days	32.5-35	35	25-28	35	8.2 ± 0.8	5.2 ± 0.4	p < 0.01
B	Normal healthy fullterms	8	1-5 days	30-35	35	22-27	×	8.5 ± 1.0	4.5 ± 0.8	p < 0.01
C ₂	Normal healthy fullterms	7	4 hrs- 6 days	30	35	30	25-27	6.4 ± 0.9	2.6 ± 0.4	p < 0.01
C ₁	Normal healthy fullterms	10	4 hrs- 6 days	35	35	35	15-22	6.6 ± 0.9	5.0 ± 0.8	p < 0.05
D ₁	Healthy prema- tures	5	< 8 days	32.5	34	27.5-29.5	×	7.1 ± 1.3	3.1 ± 0.4	p < 0.05
D ₂	Healthy prema- tures	9	> 8 days	32.5	34	27.5-30	=	17.0 ± 1.0	7.0 ± 1.0	p < 0.01

2 Circulatory response to lowering of ambient temperature, local temperature maintained constant

Table 2, Series A gives the blood flow rates in 13 fullterm infants at an ambient temperature within "neutral" zone and after a drop in ambient temperature of 4.5°-8°C. The children differed in many respects, such as age, resting blood flow prior to exposure to lower ambient temperature and the magnitude of temperature change which was tolerated until they awoke. The general trend however is quite clear. A lowering of ambient temperature of this magnitude provoked a definite decrease of blood flow (from 8.2 to 5.2 ml/min/100 ml of tissue which is a highly significant difference). The capacity to adjust the rate of blood flow through the limb according to the temperature of the surrounding air was evident already on the first day of life. This type of experiment also proves that the reduction of blood flow was a true thermogenic reflex, since any local effect on the limb circulation was ruled out by maintaining the temperature of the water in the plethysmograph at 35°C throughout the period of exposure.

3 Circulatory response to lowering of ambient temperature, local temperature not maintained constant

In order to find out to what extent the effect of a lowered temperature was due to a local effect on the skin vessels, a series of low-temperature exposures was carried out in which no efforts were made to keep local temperature in the plethysmograph at a steady level of 35°C as in Series A. The results are given in Table 2, Series B. The vasoconstriction is of the same order of magnitude as in Series A. Obviously a lowering of the local temperature adds little to the reflex response of a lowering of ambient temperature. Nor is it possible to counteract the general vasoconstrictor response by maintaining a high local temperature.

4 Circulatory response to lowering of local temperature, ambient temperature maintained constant

Two types of experiment were included in this series and the data obtained are summarized in Table 2, Series C₁ and C₂. Seven infants (Series C₁) were kept at an ambient temperature of

Table 3 The circulatory response to lowered ambient temperature on 8 premature healthy infants (local temperature not maintained constant)

No	Birth weight (g)	Gestat. period (weeks)	Post natal age (days)	Temp of incubat. (°C)	Temp of pleth (°C)	High temp blood flow (ml/min/100 ml)	Temp of incubat (°C)	Low temp blood flow (ml/min/100 ml)	Ratio	Blood flow at low temp	Blood flow at high temp
1	160	30	6	32.5	34	7.5	29	2.4		0.35	
			17	32.5	34	7.5	28	3.5		0.45	
			36	32	34	16.7	28	5.8		0.35	
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3	1930	32	22	32	34	16.9	29	3.5		0.20	
			3	32.5	34	5.0	29	3.5		0.70	
4	1500	33	7	32.5	34	11.7	29	2.2		0.20	
5	1810	35	11	33	36.5	17.4	28	6.1		0.35	
			5	32.5	34	4.5	27.5	3.4		0.75	
6	1830	37	21	32.5	34	18.7	27.5	8.8		0.45	
			9	32	34	11.6	28.5	5.4		0.45	
7	1870	36	23	32	34	12.4	30	7.3		0.60	
			21	32	34	23.0	26.5	6.8		0.30	

30°C which is at the lower limit of the assumed neutral zone. The thermal stimulus was a progressive lowering of the temperature in the plethysmograph from the initial level of 35°C to around 26°C. In this series there was a rather pronounced vasoconstrictor response from 6.4 to 2.6 ml/min/100 ml of tissue which is a highly significant difference. As the stimulus of a lowered temperature was limited to the area from which flow determinations were made there are two possible mechanisms by which a vasoconstrictor response may be obtained. In the first hand a direct effect on the cutaneous blood vessels has to be considered. And secondly there is the possibility that the reduction of blood flow is even under these experimental conditions at least partly due to a true thermoreflex over hypothalamic thermoregulating nervous structures. That the latter mechanism is quantitatively the more important is shown by the findings in Series C. Here the local cold stimulus was much stronger as the temperature of the water in the plethysmograph was gradually lowered from 35°C to 15–22°C. But despite this stronger local cold stimulus the reductions in blood flow in these infants were less prominent (from 6.6 to 5.0 ml/min/100 ml of tissue). The difference in experimental conditions in Series C₁ and C that

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5 Circulatory response in premature infants to lowered ambient temperature local temperature not maintained constant

This series was included to find out whether or not active thermoregulation is developed at premature birth. In a more detailed study on premature infants (1) we have noticed that premature infants generally have a very high rate of limb circulation under resting conditions and also that the maximal blood flow capacity at reactive hyperaemia is correspondingly higher—both studies indicating a high vascularity. We have also found that when prema-

ture infants have been examined repeatedly at various postnatal ages, the blood flow values are considerably higher in a later period, when the infant is gaining weight, than early, when body weight is still falling. Irrespective of the background to this intense peripheral blood flow and its subsequent further increase among prematures, it is obvious that these higher rates of blood flow, together with the higher surface area/bodyweight ratios in prematures are heat-loss promoting factors, which must be taken into consideration in studies on thermoregulation in prematures.

Table 3 summarizes fourteen exposures to lowered ambient temperature performed on 8 premature infants. Most of these infants were subjected to repeated examination. In Table 2, Series D₁ and D₂, the flow determinations have been grouped according to postnatal age. It is quite obvious that the general level of blood flow is markedly related ($p < 0.01$) to postnatal age.

Only a relatively small temperature change could be induced in these premature infants without producing an arousal effect. As seen from Table 3 most exposures had to be interrupted at an ambient temperature level of 28–29°C. At this temperature the infant became increasingly restless and finally woke up making any further measurements of peripheral circulation less reliable. However, before these arousal effects became prominent, limb circulation was invariably reduced by active vasoconstriction. As seen from the data in Table 3 a lowering of ambient temperature of only 3–4°C produced an average decrease of limb circulation to about 50 per cent of initial level. Thus even at advanced prematurity and irrespective of postnatal age there were no signs of impaired thermoregulatory control of cutaneous circulation.

Fig 1 illustrates the high resting blood flow in a premature infant at a postnatal age of 3 weeks. A gradual drop in ambient temperature from 32°C to 26.5°C over 2 hours brought about an extensive flow reduction to less than one third.

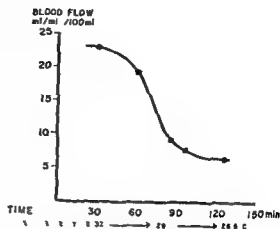


Fig 1 The circulatory response to a cold stimulus in a 3 week old prematurely born infant (gestational age 33 weeks 1420 g). Note the extensive and smooth reduction in blood flow of the foot and calf. See also Table 3.

DISCUSSION

The present report is based on a large number of blood flow measurements at different temperature conditions. The data obtained show that the rate of circulation in the foot and calf is highly dependent on the temperature to which the child may be exposed. Any deviation from the neutral zone immediately brings about a reflex change of peripheral circulation by which heat loss from the body surface is adjusted to the altered temperature of the child's surroundings. This circulatory adaptation has been shown to be a true thermoregulatory reflex, since in the present study the local temperature at the area from which the blood flow records were obtained could be maintained constant. The local effect of cooling must be relatively less important since it was largely abolished if ambient temperature was kept high. It should also be stressed that the reductions in peripheral circulation that may be achieved are quantitatively important.

The present study also confirms our previous observations (3, 6) that in the first hours of life the sympathetic tone on peripheral blood vessels is higher than later in the neonatal period. At this early age the responsiveness to thermal stimuli is also less prominent. The probable explanation is that as important adaptive processes are still going on the vas-

cular bed may be preoccupied with other central or reflex nervous influences over the vasomotor nerves. The vast vascular supply that characterizes the premature infant will be discussed elsewhere (1). In this connection we would only like to stress the high basal blood flow which is typical for the premature infant in the later phase of increasing body weight. Not only has such a premature infant an unfavourable surface to body mass ratio; the blood flow per unit surface must also be considerably bigger.

The effect of a colder environment on circulation is by no means limited to a decrease of volume flow through the extremities. The plethysmographic technique is basically a method to register volume and volume changes. We have noticed repeatedly that exposure to a colder environment is followed by a measurable decrease of limb volume indicating that the capacity function of the vascular bed is also interfered with.

Our studies were confined to sleeping infants and were interrupted as the infants woke up. However it was quite clear that arousal is part of the effect of a colder environment on the infant as a whole. The critical temperature at which arousal became prominent was in the majority of cases around 27-29°C. At higher temperatures 31-34°C most infants were at thermal comfort. Few children tolerated a temperature of 22-23°C without being awakened. It would perhaps be possible to induce a greater temperature change than this provided this change was more rapid. The behaviour at the arousal from sleep at thermal comfort was quite typical. The first sign was an increase of flexor tone combined with short flexor movements. During progressive exposure to a colder environment this increase of muscular activity was accompanied by gross muscular movements and bouts of crying for 10 to 30 sec. The infant then became calm again for 1 to 2 min after which new series of increasing muscular activity appeared with increasing frequency. Definite shivering was not observed but the described pattern of

movements should probably be looked upon as an equivalent to shivering, and therefore as an integral part of thermoregulation at early age.

The present observations on the rate of peripheral blood flow and the previous findings by Bruck and co-workers (2) on skin temperature show that the lability of body temperature well known for newborns and especially pronounced for the prematurely born cannot be attributed to a non functioning thermoregulatory control of peripheral circulation. Other factors such as a large body surface being richly vascularized, poor insulation by subcutaneous fat, short distances from the surface to core and a relatively larger insensible perspiration may account for greater heat losses when the newborn is contrasted against the adult. Likewise recent evidence (2, 8) shows that thermogenesis too is a prominent part of the thermoregulating mechanism in the newborn. Active thermogenesis in the newborn is probably largely restricted to skeletal muscle. The simple fact that skeletal muscle comprises a far smaller proportion of body mass in the newborn (and especially in the premature) than in the adult may pose another handicap for the newborn.

SUMMARY

Blood flow measurements by venous occlusion plethysmography were performed on 131 sleeping healthy fullterm newborns at various external temperature conditions. The peripheral circulation varies among different individuals but a definite relationship between ambient temperature and the rate of peripheral circulation could nevertheless be established.

Different series of observations on exposure to acute changes of temperature conditions on a further 46 newborn infants allow the following conclusions.

The control of peripheral circulation in relation to the temperature of the surroundings is reflex in nature and independent of the local effects of a cold stimulus.

Circulatory changes are quantitatively pro-

ture infants have been examined repeatedly at various postnatal ages, the blood flow values are considerably higher in a later period, when the infant is gaining weight, than early, when body weight is still falling. Irrespective of the background to this intense peripheral blood flow and its subsequent further increase among prematures, it is obvious that these higher rates of blood flow, together with the higher surface area/bodyweight ratios in prematures are heat-loss promoting factors, which must be taken into consideration in studies on thermoregulation in prematures.

Table 3 summarizes fourteen exposures to lowered ambient temperature performed on 8 premature infants. Most of these infants were subjected to repeated examination. In Table 2, Series D₁ and D₂, the flow determinations have been grouped according to postnatal age. It is quite obvious that the general level of blood flow is markedly related ($p < 0.01$) to postnatal age.

Only a relatively small temperature change could be induced in these premature infants without producing an arousal effect. As seen from Table 3 most exposures had to be interrupted at an ambient temperature level of 28–29°C. At this temperature the infant became increasingly restless and finally woke up making any further measurements of peripheral circulation less reliable. However, before these arousal effects became prominent, limb circulation was invariably reduced by active vasoconstriction. As seen from the data in Table 3 a lowering of ambient temperature of only 3–4°C produced an average decrease of limb circulation to about 50 per cent of initial level. Thus even at advanced prematurity and irrespective of postnatal age, there were no signs of impaired thermoregulatory control of cutaneous circulation.

Fig. 1 illustrates the high resting blood flow in a premature infant at a postnatal age of 3 weeks. A gradual drop in ambient temperature from 32°C to 26.5°C over 2 hours brought about an extensive flow reduction to less than one third.

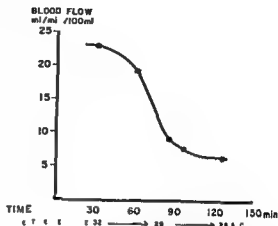


Fig. 1 The circulatory response to a cold stimulus in a 3 week-old prematurely born infant (gestational age 33 weeks 1420 g). Note the extensive and smooth reduction in blood flow of the foot and calf. See also Table 3.

DISCUSSION

The present report is based on a large number of blood flow measurements at different temperature conditions. The data obtained show that the rate of circulation in the foot and calf is highly dependent on the temperature to which the child may be exposed. Any deviation from the neutral zone immediately brings about a reflex change of peripheral circulation by which heat loss from the body surface is adjusted to the altered temperature of the child's surroundings. This circulatory adaptation has been shown to be a true thermoregulatory reflex, since in the present study the local temperature at the area from which the blood flow records were obtained could be maintained constant. The local effect of cooling must be relatively less important since it was largely abolished if ambient temperature was kept high. It should also be stressed that the reductions in peripheral circulation that may be achieved are quantitatively important.

The present study also confirms our previous observations (3, 6) that in the first hours of life the sympathetic tone on peripheral blood vessels is higher than later in the neonatal period. At this early age the responsiveness to thermal stimuli is also less prominent. The probable explanation is that as important adaptive processes are still going on the vas-

NIEMANN PICK DISEASE AND GIANT CELL TRANSFORMATION OF THE LIVER

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Doubts have grown as to how many genotypically different syndromes are in reality encompassed by Niemann Pick disease. The biochemical disturbance in over 160 recorded cases has been broadly classified as a *sphingomyelin sterol lipidosis* ■ cause of the accumulation of sphingomyelin together with cholesterol and glycerophospholipids predominantly in tissue foam cells within the reticuloendothelial system. There is however a broad range in the severity and distribution of such accumulations apart from great variations in the age of onset and clinical evolution with special reference to the timing and degree of CNS involvement. But to what extent this heterogeneity of clinical and biochemical expression represents true genotypic variation remains to be resolved.

The first of the four clinico-pathological subdivisions of Crocker (3) is a rapidly progressive infantile form believed to typify 85% of all the victims. A classical pattern comprises an onset in early infancy ending in death by the age of 1 to 3 years, a high incidence amongst Jews, massive hepatosplenomegaly, intense CNS involvement, and an association with an especially high sphingomyelin content of involved tissues. Within this category examples

of particularly early manifestations have presented themselves. Infiltration was already grossly identifiable in the foetus in two examples (2, 12) and has been recognizable at birth or during the first month in more than 12 cases.

Even amidst those already involved pre- and neonatally the three affected siblings we document here may be representative of yet another specific grouping. Neonatal jaundice emerging by the 10th day was their prominent differential feature. It can also be identified in the proforma of the two sibs of Maurer (15), three sibs of Jeune et al (10), three sibs of Crocker & Farber (3) and two of Ivemark et al (8). In one of the three examples cited by Frederickson (4) jaundice persisted from shortly after birth whereas the other two did not quite conform in that neonatal jaundice lingered only 4 weeks in one and disappeared after a few days to reemerge after 7 weeks in the other. It may be noted moreover that in addition to the background of consanguineous marriage in our cases and those of Crocker & Farber (3) and Ivemark et al (8) all cases presented with neonatal jaundice were in familial groups although the overall familial incidence for all cases of Niemann Pick was calculated by Frederickson as not exceeding 25%. Likewise although he assessed the Jewish incidence of this disease at about 40% all the familial groups hitherto reported in our category had

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minent and do not support the view that thermolability in the newborn is due to a deficient thermocontrol of the cutaneous circulation

The circulatory thermoreflex operates even during the first day of life and is as prominent in premature infants as in fullterm infants

The importance of these observations in relation to other mechanisms deciding heat loss and thermogenesis in the newborn is briefly discussed

ACKNOWLEDGEMENT

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the jaundice deepened, the child remained active with normal reflexes posture and psychomotor development. The weight gain was slow the abdomen was distended mainly because of the liver which extended 5.5 cm below the right costal margin (Fig 1)

Laboratory findings The serum bilirubin both direct and indirect, was estimated throughout the course of the illness (Fig 2). It can be seen that the bilirubin level was 18 mg/100 ml admission rising to 23 mg/100 ml within a few days. On prednisone therapy the serum bilirubin decreased to 15 mg/100 ml. When the prednisone was reduced to 40 mg/day the level rose again to 21 mg/100 ml a fall to 15 mg/100 ml following upon a return of the prednisone dosage to 60 mg/day. On tapering off the steroid administration the bilirubin rose gradually and before operation was 32.8 mg/100 ml.

The SGOT on admission was 162 and gradually rose to 240 units the SGPT on admission was 10 rising gradually to 0 and then more sharply to 146 units. Total protein ranged between 5.4-6.8 mg/100 ml. Serum cholesterol was between 195 and 284 mg/100 ml. Thymol turbidity on admission was 1.8 units the thymol flocculation was negative and cephalin flocculation was ++ later on the thymol turbidity rose to 9.2 units, thymol flocculation to ++++ whereas the cephalin flocculation remained ++.

Hypochromic anaemia developed during the course of the illness with the haemoglobin declining from 11.4 to 7.4 g/100 ml. Leucopenia was not prominent nor was there thrombocytopenia. Leucocyte counts were between 7 100 and 4 950/mm³ thrombocytes 760 000/mm³. No Niemann Pick cells were detected in the peripheral blood smears nor in the bone marrow.

An exploratory laparotomy was performed at which a biopsy was taken from the liver. This was examined histologically electron microscopically and biochemically.

Frozen and paraffin embedded sections were examined. The liver showed a distortion of the lobular pattern and an occurrence of many parenchymatous giant cells. There were bile casts in the canaliculi and pigment within the giant cells. Some liver cells were swollen and so were some sinusoidal cells. There was an increase in the fibrous tissue in portal areas and in between the liver cells. Bile ductules and ducts in portal areas appeared scanty in number.

A lipodosis was not suspected, and only on re-examining the slides after the baby's death, was it realised that the sinusoidal cells were indeed lipid laden Niemann Pick cells.

Electron microscopic examination showed the liver cells to contain many myelin figures and lobulated aculeate like structures (Fig. 10) (19).

Enzyme studies of the biopsy material showed marked decrease of glucuronyl transferase activity. UDPG glucuronyl transferase (EC 2.4.1.17) was assayed with 4-methylumbelliferone as substrate by a modification of the method of Arias (1). Glucose-6-phosphatase was assayed according to Hers (6). Glycogen was determined by an enzymatic method em-

ploying amyloglucosidase and glucose oxidase (9) after alkaline hydrolysis and precipitation with ethanol.

RESULTS

	Glucuronyl transf (nmol/mg prot/min)	Normal
Case 1		
Liver biopsy	1.12	87 ± 0.6
Liver PM	0.64	
Kidney PM	5.02	
Case 3		
Liver biopsy	0.99	

Liver glycogen concentration in the biopsy obtained from case 1 was 1.2%. In the case of case 3 another microsomal enzyme glucose-6-phosphatase was also determined. Activity of this enzyme was 57 nmol/mg prot/min (controls 39 ± 2).

The low activity of liver glucuronyl transferase was in contrast to the normal activity found in the kidney of case 1 and could not be related to the apparently unimpaired ability of the liver to conjugate bilirubin as evidenced by the elevated serum level of direct reacting bilirubin.

In vitro inhibition of UDPG glucuronyl transferase by bilirubin has recently been described but it seems improbable that such an inhibition was the cause of the low enzyme activity observed (5) the concentrations at which bilirubin inhibited the reaction were extremely high whereas in our assay the final concentration of bilirubin in the reaction mixture did not exceed 0.5 mg/100 ml. Results obtained with different amounts of homogenate in the assay giving final bilirubin concentrations ranging from 0.5 mg/100 ml were identical.

The question whether there exists only one enzyme glucuronidating the various substrates used for assay of UDPG glucuronyl transferase in addition to bilirubin has not been finally settled.

Although quantitative differences in the activity of human liver enzyme towards various



Fig 1 Case 1 At the age of 2 months showing enormous enlargement of liver and spleen

been described as non Jewish. Of other components shared, at least in our case and those of Crocker and Ivemark, an interesting aspect is the hepatic giant cell transformation which in several instances led indeed to initial confusion with giant cell hepatitis and cirrhosis because of the coexisting fibrosis and cholestasis.

The relationship of jaundice would be worthy of further study. Despite previous claims of full functional normality, serial measure of the transaminase especially of SGOT, revealed early evidence of parenchymal cell damage or dysfunction. Our parallel finding of

almost absent glucuronyl transferase activity in the liver biopsy material could account, however, for only a small proportion of the early and persistent jaundice, since the total serum bilirubin in our infants embodied a relatively high proportion of the direct or conjugated form (Fig 2).

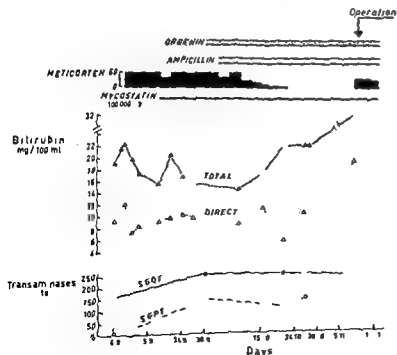
DESCRIPTION OF CASES

Family history. The parents are Persian Jews related to each other, the father being the mother's uncle. Both are healthy with no past history of serious illness or jaundice. Their first child was born in 1964, a normal healthy girl who is now 8 years old. The second child, also a girl (our case 2), was born in 1965 and died at the age of 3 months at another hospital. The third child, a girl, was born at term in August 1966 and is our case 1.

In 1969, a full term boy (case 3) was born and developed a clinical picture similar to the two previous siblings: liver biopsy again showing sphingomyelin lipidosis and giant cell transformation. This child also died at age of 3 months and no permission for autopsy could be obtained.

Case 1

Clinical picture. A girl weighing 2700 g was born at term after a normal pregnancy. The immediate neonatal period was uneventful except for the appearance of jaundice on the second day of life, which increased for 2 days and disappeared by the sixth day of life, only to reappear 8 days later in association with yellowish brown urine and pale stools. Although



the jaundice deepened the child remained active with normal reflexes posture and psychomotor development. The weight gain was slow the abdomen was distended mainly because of the liver which extended 5.5 cm below the right costal margin (Fig. 1).

Laboratory findings. The serum bilirubin both direct and indirect, was estimated throughout the course of the illness (Fig. 2). It can be seen that the bilirubin level was 18 mg/100 ml admission rising to 23 mg/100 ml within a few days. On prednisone therapy the serum bilirubin decreased to 15 mg/100 ml. When the prednisone was reduced to 40 mg/day the level rose again to 21 mg/100 ml a fall to 15 mg/100 ml following upon a return of the prednisone dosage to 60 mg/day. On tapering off the steroid administration the bilirubin rose gradually and before operation was 32.8 mg/100 ml.

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Fig 3 Case 1 Liver form cells giant cells and interstitial fibrosis H and E $\times 147$

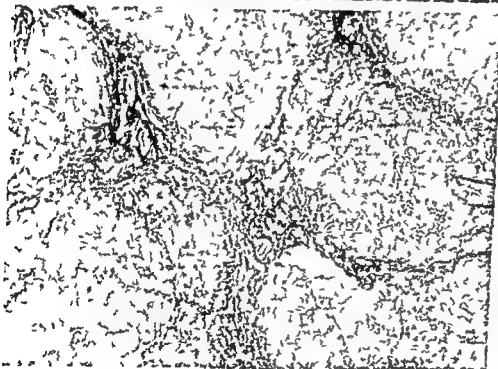


Fig 4 Case 1 Area of liver cirrhosis Azan $\times 43$

substrates have been observed results with 4 methylumbelliferone have been in line with those obtained with O aminophenol or bilirubin as acceptor (1). The main difference between the assay with 4 methylumbelliferone and other substrates was in the greater sensitivity of the former, for this reason trace activities were obtained with umbelliferone in cases in which no

activity could be detected with other substrates (1).

Activation of UDPG glucuronyl transferase activity towards various substrates by *in vitro* treatment of enzyme preparations with detergents has recently been described (13). It has been proposed that addition of detergents and other procedures causing *in vitro* activa-



Fig 5 Case 1 Thymus almost unrecognisable because of lipid deposition within cells H and E $\times 43$

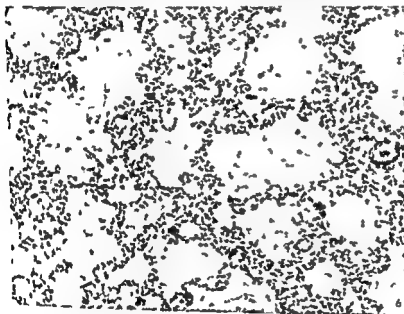


Fig 6 Case 1 Lungs with foam cells in alveoli: H and E, $\times 147$

tion (7) lead to an unfolding of microsomal membranes which makes the substrate accessible to the enzyme (16). If this is the case the low enzyme activity found in the present cases may possibly be due to changes in the cell structure connected with the underlying disease which make the enzyme less accessible to the substrate. Such a phenomenon in a crude

non activated homogenate would not necessarily reflect the *in vivo* situation and explain the apparent discrepancy between the unimpaired ability to conjugate bilirubin *in vivo* and the low *in vitro* enzyme activity observed.

Although there was no bleeding tendency the baby developed haemorrhagic ascites and died 5 days after the operation aged 3 months.

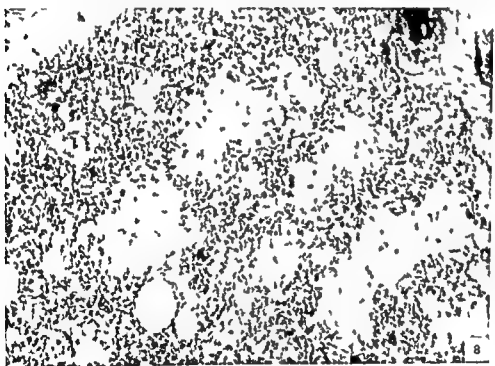


Fig 7 Case 2 Thymus with lipid deposition in cells H and E $\times 108$

Fig 8 Case 2 Lungs with foam cells in alveoli H and E $\times 108$

Autopsy

Gross findings The peritoneal cavity contained 80 ml of bloody fluid and some clotted blood around the site of the operative liver biopsy. The liver was enlarged, firm and greenish in colour, its surface and sections were unevenly granular. The extra hepatic bile ducts were patent and normal, the gall bladder was rather small and contained a small amount of yellowish

viscous fluid. The spleen weighed 120 g, was fairly firm, dark red in colour with vaguely defined paler areas. The lymph glands and thymus were small. The lungs were well aerated but had near their periphery small, dark red consolidated areas. The other organs, including the brain, showed no gross abnormalities.

Microscopic findings Formalin and Zenker fixed tissue blocks were first examined. When

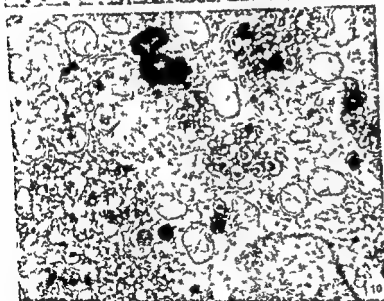


Fig 9 Case 2 Liver with giant cells and fibrosis H and E, $\times 147$

Fig 10 Electron micrograph of the liver biopsy of case one. The changes are non specific. There are lobulated vacuolelike structures in the parenchymal cells (19)

it became obvious that the condition was a lipidosis, the stored organs were transferred to Baker's solution after 2 months in 10% formalin and frozen tissue sections were used for the various lipid stains.

The liver Numerous sections were examined and these presented varied appearances. Some areas preserved a fairly normal liver architecture showing only an increase in connective tissue in portal areas and between liver cords and marked canalicular and intracellular bile stasis.

These areas had normal bile ducts of various sizes in the portal tracts. In other areas there was a marked giant cell transformation of liver cells with total loss of normal architecture and paucity of bile ducts. The sinusoidal cells as well as some liver cells were often foamy too (Fig. 3) so that it was difficult at times to distinguish between them. In many regions definite cirrhosis with regenerative nodules could be seen (Fig. 4).

The reticuloendothelial system The spleen,

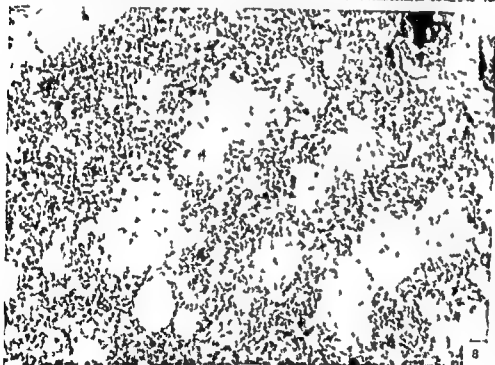


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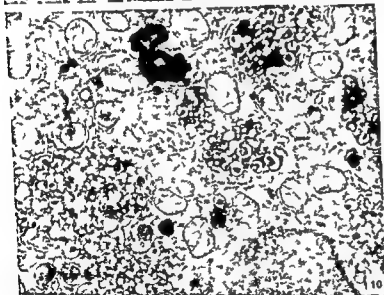
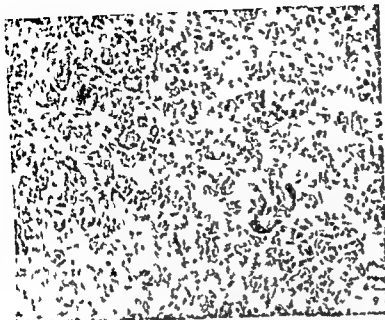


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The reticuloendothelial system The spleen

Table 1 Results of histochemical procedures compared with the reactions usually obtained in Niemann Pick disease (14, 20)

- = negative ± = slightly positive or variable + = positive ++ = strongly positive ND = not done

	Case 1			Case 2			Niemann Pick disease
	Lung	Liver	Spleen + thymus	Lung	Liver	Spleen + thymus	
Sudan red (with heating)	±	±	-	-	+	-	±
Sudan black	++	++	++	++	++	++	++
Schultz method for cholesterol	+	ND	+	+	ND	±	+
Nile blue for fatty acids	+	ND	+	ND	ND	ND	+
Luxol blue (myelin stain)	±	+	-	ND	ND	ND	±
Smith-Dietrich method for phospholipids	++	++	++	ND	ND	ND	+
Baker's procedure for phospholipids (with pyridine extraction)	++	++	++	++	++	++	+
Toluidine blue metachromasia (PH7)	ND	+	+	ND	+	+	±
PAS + Diastase	ND	+	±	ND	±	±	±

thymus, lymph glands, bone marrow and Peyer's patches contained large numbers of typical Niemann-Pick cells. The thymus was almost totally replaced by foam cells (Fig. 5).

The lungs In several areas typical Niemann-Pick cells filled the alveoli (Fig. 6). Lipid-laden cells also occurred occasionally within the septa.

Foam cells were also found in many kidney glomeruli at junction of cortex and medulla of the adrenals and in the lamina propria of the intestines.

The central nervous system Many neurons in the spinal cord, brain stem and basal ganglia were ballooned, with foamy cytoplasm and indistinct cellular borders. These, however, stained negatively for lipids.

Case 2

A sister of case 1 died 1 year previously at another hospital also at the age of 3 months after a very similar clinical course. The jaundice appeared at the age of 10 days, deepened gradually alongside with progressive hepatosplenomegaly. The post mortem diagnosis were viral giant cell hepatitis, interstitial pneumonia and malnutrition. The formalin-fixed organs of this case were available for renewed study.

Microscopic examination of the spleen, lymph nodes, thymus and lungs showed large numbers of typical foam cells (Figs. 7 and 8). The liver presented parenchymatous giant cell transformation, fibrosis and foamy Kupfer cells (Fig. 9).

In spite of the prolonged formalin fixation (18 months) it was possible to demonstrate pronounced

lipid storage and a positive Baker's test for phospholipids in the liver, thymus, lymph glands, spleen and lungs. That this too was a case of Niemann-Pick disease together with a giant cell transformation of the liver.

The histochemical tests performed in both cases as well as the reactions usually obtained in Niemann-Pick disease are summarised in Table 1.

DISCUSSION

Especially striking clinico-pathological analogies can be drawn between the three siblings herewith described and the two siblings of Ivemark (8). But such a resemblance, albeit less complete, can readily be extended to the familial groups of Maurer (15), June (10), Crocker & Farber (3), and Frederickson (4). A very early postnatal onset almost certainly following already prenatal involvement was highlighted by their neonatal jaundice. This persisted and was marked by a sustained high level of conjugated or direct serum bilirubin. The infantile course was fulminant with early and progressive hepatosplenomegaly underlying typically conspicuous abdominal protuberance. This was also attended by feeding difficulty with recurrent vomiting and an overall failure to thrive. Death usually ensued within 3 to 9 months.

Pathologically, another impressive analogy lay in the accompanying hepatic giant cell

transformation fibrosis and cholestasis inviting a firm preliminary diagnosis of viral giant cell hepatitis in several examples. The initial clinical expression of the familial form of Niemann Pick, with its early and conspicuous hepatic component usually sufficed to overshadow the clinical picture and conceal the true nature of the disorder.

Add to this laboratory evidence of parenchymal liver damage as well as cholestasis and the diagnostic diversion could be complete. Significantly abnormal liver function tests are usually expected however only in terminal phases. With respect to thymol turbidity and cephalin flocculation tests this was also true at least of our case 1. But of the serum transaminases whilst SGPT levels were not raised the SGOT was clearly increased from an early stage. Strangely enough this particular disparity between the serum transaminase findings has previously been noted in several examples of another storage disorder Tay Sachs disease (17).

Electron microscopy of liver biopsy material may clarify the underlying diagnosis but specifically oriented lipid histochemistry can also readily identify the sphingomyelin sterol laden foam cells in various organs. With regard to either the Niemann Pick disorder or hepatic giant cell transformation there has been evidence that severe involvement can already arise prenatally even perhaps dating back to the first trimester. But rather than suggesting a primary and even associated genetically-determined disturbance it is increasingly suspected that hepatic giant cell transformation expresses a non specific reaction characterizing the immature parenchyma of the developing liver (4, 8, 11, 18). In our siblings, as well as in those of Iwemark et al (8) it presumably reflected a secondary response to the concomitant Niemann Pick defect.

Lung involvement is also uniformly typical of this disease. Foam cells may fill the alveoli although there is nonetheless disproportionately slight impairment of respiratory function. The radiological stigmata of a diffuse reticular or

of a widespread nodular pattern may suggest a chronic interstitial pneumonia, as was both the pre and post mortem interpretation in the second case. A helpful diagnostic measure could be the analysis of gastric aspirates for pathognomonic foam cells.

SUMMARY

This report presents three siblings with neonatal jaundice who died before the age of three months. They were shown on autopsy to be suffering from Niemann Pick disease, together with a giant cell transformation of the liver.

The resemblance of these cases to previously reported examples of the above mentioned double condition is stressed and a suggestion is made that this variant of the Niemann-Pick disease presents a special genotypic syndrome within the group with the biochemical disorder broadly classified as sphingomyelin lipidosis.

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CARDIOMEGALY IN ASSOCIATION WITH NEONATAL HYPOGLYCAEMIA

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Hypoglycaemia is a well recognised complication in infants of low birth weight in relation to gestational age and in infants of diabetic mothers in the immediate neonatal period. Various symptoms have been described occurring in association with hypoglycaemia apnoea convulsions lethargy and difficulty with feeding (4).

In the Hospital for Sick Children Toronto Dr George Collins (now in Montreal) first brought to our attention the occasional association of heart failure with neonatal hypoglycaemia. Dr Bernard Reilly independently noted the same association with increased radiological heart size and pulmonary oedema.

The purpose of this paper is to report the incidence and clinical course of this condition over a 12 month period between February 1968 and February 1969. We have found 6 infants who had hypoglycaemia tachypnoea (respiratory rate $>65/\text{min}$) and radiographic cardiac enlargement and signs suggestive of pulmonary oedema unassociated with other organic disease.

MATERIAL AND METHODS

The Neonatal Division of the Hospital for Sick Children, Toronto dealt with more than 1000 patients

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during the above mentioned period of these, 550 were admitted to the Intensive Care Unit within the Division. These are referred from maternity units in the Toronto area, the majority suffering from respiratory disease in association with low birth weight, the remainder having congenital heart disease metabolic disorders or neonatal surgical conditions (9). During this period hypoglycaemia was diagnosed in 52 infants. We found 13 infants with isolated symptomatic hypoglycaemia, of whom 6 had cardiomegaly in association with the hypoglycaemia. None of the 13 infants had any of the following conditions: respiratory distress syndrome, transient tachypnoea of the newborn (?), congenital heart disease, sepsis, anaemia or polycythaemia (3), all of which may be associated with cardiac enlargement. None of the infants had Apgar scores less than 5 and delivery was normal or by low forceps.

Blood samples for sugar determination were obtained by heel prick or venous puncture on admission and thereafter at varying intervals depending on the infant's condition and the initial result. The sampling was carried out every 2 to 6 hours until normal levels were attained.

The blood sugar was measured by a modification of the method (8) using the reduction of potassium ferricyanide to potassium ferrocyanide by glucose.

Hypoglycaemia was diagnosed when the blood sugar was reported as being $<25 \text{ mg}/100 \text{ ml}$ in the first 24 hours of life and $<30 \text{ mg}/100 \text{ ml}$ after that period.

It is acknowledged that measurement of the cardiothoracic ratio on an AP film rather than PA film with the patient recumbent rather than upright, and at a focal film distance of 40 inches rather than 72 inches tends to vitiate the accuracy of this measurement as an absolute value. However as the circumstances were the same in all cases, the cardiothoracic ratio (C/T) can be considered a valid way of estimating change in heart size. The cardiac enlargement was noted independently of the clinical findings, and diagnosed on comparison with a large volume of neonatal chest films.

Table 1 Symptoms and X ray findings of cases 1-6

No	Sex	Gestation (week)	B W (g)	Age at onset of symptoms (hr)	Symptoms	X ray chest		
						Initial and final C/T ratio	Pulm oedema	Diagnosis
1	♂	39	2 300	5	Apnoea	61-51	+	Dysmaturity
2	♂	40	2 580	24	Jittery Tachypnoea	63-47	+	Dysmaturity
3	♂	39	2 180	7	Jittery Lethargy Cyanotic spells Tachycardia Tachypnoea	68-56	+	Dysmaturity
4	♂	36	1 450	36	Tachypnoea	66-55	+	Dysmaturity
5	♂	38	1 920	8	Jittery Tachypnoea	57-46	+	Dysmaturity
6	♀	36	1 900	46	Jittery Tachypnoea	62-51	+	Dysmaturity

RESULTS

There were 6 infants (Table 1) of the 52 with hypoglycaemia who also had radiographic cardiac enlargement. This ranged in severity from a marginally normal cardiothoracic ratio of 57% to a grossly abnormal 68% (Fig 1). All the cases also exhibited pulmonary oedema of varying degree, the most severe having a small pleural effusion. In addition to the classical signs of symptomatic hypoglycaemia, 5 of the 6 infants had tachypnoea (respiratory rate > 65/min) and one, symptoms suggestive of conges-

tive heart failure (Case 3) as manifest by tachypnoea, tachycardia, and hepatomegaly. No crepitations were heard over the lung fields, however, interstitial pulmonary oedema is notably lacking in this physical sign unless true alveolar flooding is present. In every case the clinical signs and symptoms disappeared on correction of the hypoglycaemia. There was no doubt at all that the heart originally large became smaller and the pulmonary oedema disappeared (Fig 2). The course of one of the infants (Case 3) is shown in Fig 3a and b the films being taken at the age of 60 and 90 hours.

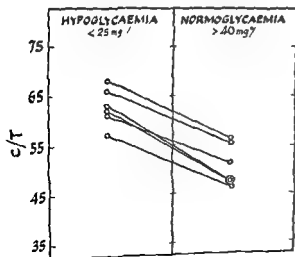


Fig 1 Cardiothoracic ratio (C/T)—Cases 1-6

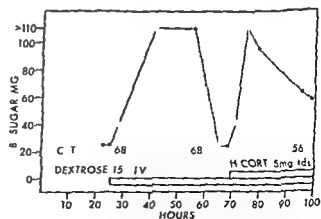


Fig 2 Case 3 demonstrating blood sugar levels and cardiothoracic ratio (C/T)

The 6 infants were small for gestational age using the criteria of Usher & McLean (12). Electrocardiographs were taken in Cases 1-4 and were within normal limits for newborn infants.

As previously noted above there were 7 further infants who were comparably hypoglycaemic for approximately the same period of time but had no cardiac enlargement. Six of these were small for gestational age and all had hypoglycaemic symptoms related to the central nervous system and received active therapy. All 13 infants had similar prenatal, natal and postnatal histories. Their serum electrolytes and haematocrits were within normal limits (Table 2).

Table 2 Apgar score, haematocrit, method of delivery and ECG findings

Case no	Apgar score	Haematocrit	Method of delivery ^a	ECG findings
1	6	58	N/D	Normal
2	9	66	L/F	Normal
3	5	62	L/F	Normal
4	7	60	N/D	Normal
5	5	59	M/F	Not done
6	10	60	N/D	Not done

N/D = Normal delivery

L/F = Low forceps

M/F = Mid forceps

DISCUSSION

Radiological (cardiac enlargement, pulmonary oedema) or clinical (tachypnoea, tachycardia, hepatomegaly) signs suggestive of cardiac failure resolving on treatment of hypoglycaemia have not previously been described.

The incidence of symptoms in hypoglycaemia varies according to different authors (4-10). The majority of the symptoms relate to the central nervous system and vary from lethargy to convulsions.

Since cardiac enlargement disappeared upon the attainment of normoglycaemia, we would suggest that the cause of the heart failure may be related to insufficient cardiac energy substrates, the body content of both glycogen and lipid being deficient in dysmature infants (1-10).

Yao and co-workers (13) have shown that small for date infants may have increased blood red cell and plasma volumes compared with other newborn infants. We have no data on these values on our cases, although the haematocrit levels were within normal limits. It is therefore possible that some part of the cardiac failure has been due to circulatory overloading. However, the close correlation between the radiological and clinical findings and the blood sugar levels were still very striking.

It is important to recognise this manifestation of hypoglycaemia in order that it can be



Fig 3 a and b X-ray findings during and after period of hypoglycaemia in Case 3

rapidly corrected, if necessary with corticosteroids (6). In this way the period during which the infant has signs and symptoms of central nervous system and cardiovascular system is minimised. The condition should be considered in the differential diagnosis of cardiomegaly of heart failure in the newborn period.

In the light of our finding of the deleterious effects on the cardiovascular system and of others suggesting the possibility of hypoglycaemic damage to the central nervous system (5), we cannot agree with the suggestion of Griffiths (7) based on the post mortem findings in 22 infants with hypoglycaemia, that treatment may be unnecessary.

SUMMARY

Fifty-two infants with hypoglycaemia were seen over a 12-month period. Six of these infants had cardiac enlargement with signs of failure and pulmonary oedema in association with isolated hypoglycaemia. Signs and symptoms suggestive of cardiac failure corresponded to the period of hypoglycaemia and disappeared when blood sugar levels were raised by appropriate treatment.

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URINARY GLYCOSAMINOGLYCAN EXCRETION IN THE NEONATAL PERIOD

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An increase in urinary glycosaminoglycans (GAG) is a useful diagnostic feature of the hereditary mucopolysaccharidoses. However, there is little available information on the excretion of these substances in the early weeks of life when there is difficulty in obtaining 24-hour samples of urine.

In this laboratory a cetylpyridinium chloride (CPC) turbidity test is used as a semiquantitative screening test for excess GAG excretion (4). Although this test has proved useful in older children (5), the lack of adequate information on the normal GAG excretion by neonates makes it difficult to interpret screening tests in such subjects. The same applies to the CPC precipitable uronic acid which is usually measured when a screening test is positive. Inspection of published data shows that only two of the normal children studied by Teller et al. (6) and only five of those studied by Manley et al. (3) were less than three months old.

In view of the difficulties concerning both 24-hour urine collections and the interpretation of uronic acid excretion, this investigation was planned to determine the range of normal CPC precipitable uronic acid in random urine samples collected from infants under six months of age.

MATERIALS AND METHODS

Random samples of urine were collected from 160 normal neonates (birth weight 3-4 kg, gestation 37-

42 weeks) while they were in the newborn nursery at Southmead Hospital, Bristol, or at various intervals after discharge from hospital. A further 30 samples were collected from infants aged 3-6 months.

The CPC precipitable GAG were isolated by the method of Di Ferrante (7) using reduced volumes where necessary, and the uronic acid content was estimated by the method of Bitter & Muir (1). Urinary creatinine was estimated using an autoanalyser method (Technicon method file N 11).

RESULTS

The CPC precipitable uronic acid excretion expressed as mg per g of creatinine is shown in Fig. 1. Results on six samples were excluded as the creatinine concentration was less than 5 mg per 100 ml and an error of only 1 mg either side of this figure would markedly affect the ratio. The normal range is depicted by the lines drawn by inspection as there were insufficient samples at certain ages to adequately undertake a statistical analysis. There was no apparent difference between males and females. Thirty samples from infants aged three to six months gave a mean uronic acid result per g of creatinine of 20.5 ± 8.9 (S.D.).

DISCUSSION

The CPC citrate screening test (4) gives approximately 3% positive results which require further investigation in children over one year of age (5). However, if 300 CPC units per g creatinine is accepted as the upper limit of

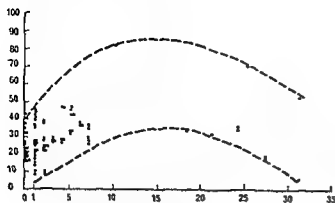


Fig. 1 Showing uronic acid excretion per g of creatinine against age in days

normal up to six months of age, we find a large number of positive results in neonates. When CPC precipitable uronic acid is estimated, many samples give a result above the normal upper limit of 32 mg per g creatinine at six months of age given by Teller et al (6). Our results in normal infants aged three to six months are in close agreement with theirs. However, the results presented here show that the excretion of GAG as reflected by CPC precipitable uronic acid can be above this upper limit in neonates.

Thus GAG excretion expressed per g creatinine is higher in the neonatal period than at any other time in life. There is an increase from birth reaching a peak in the second week and thereafter declining throughout childhood to adult levels.

We believe that a screening test which takes urine concentration into account is essential at this age since only random samples of urine are easily obtained. In our screening test (4) we still retain an upper limit of normal at 300 CPC units/g creatinine for samples from infants below the age of six months. We prefer to do uronic acid estimations on samples giving results above this because we would be less likely to miss a genuine positive result under these conditions and the additional work load of analysing false positives is not excessive.

The early diagnosis of the mucopolysaccharidoses is essential if genetic counselling is to be part of the management of this group of disorders. The fact that glycosaminoglycan excretion, expressed as CPC precipitable uronic

acid per g of creatinine, is high in the neonatal period may make early diagnosis difficult at this time if reliance is placed solely on urinary excretion studies.

SUMMARY

Urinary glycosaminoglycan excretion relative to creatinine is high during the neonatal period reaching a peak in the second week of life and thereafter declining throughout childhood. The difficulty in early diagnosis of the mucopolysaccharidoses during the neonatal period is emphasized.

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INHIBITION OF ERYTHROPOIESIS BY PLASMA FROM NEWBORN INFANTS

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Several investigators have previously stressed the marked and rapid change of erythropoiesis in the normal newborn infant. During the first week of life the reticulocyte count decreases from high levels to normal or below normal adult levels (23-24). While the erythropoietin levels are slightly elevated in cord blood no erythropoietin is detectable in plasma from infants during the first weeks of life (9). The rapid decrease of erythropoiesis can be due to disappearance and/or utilization of erythropoietin alone but an inhibitory mechanism may also be involved (10). An analogous condition can be found in high altitude natives brought to sea level. A decrease of erythropoiesis occurs and an inhibiting effect of plasma from such persons on erythropoiesis in recipient animals has been described (4, 19, 20, 21). Several authors have reported the presence of erythropoiesis inhibiting factors in plasma from hypertransfused animals as well (12, 19, 26, 27) while others have been unable to confirm this (3, 16).

These findings raise the following questions: Does an inhibiting factor participate in the depression of erythropoiesis in the newborn infant? If so from which day of life and for how long time is this factor detectable? The present paper reports investigations to answer these questions.

MATERIAL

Normal infants

Cord blood was collected from 7 infants born after normal pregnancies and with normal deliveries. Blood

(4-10 ml plasma) was further collected by venesection from 11 normal infants between 2 and 84 days of life. Three of these infants had been admitted to the department because previous siblings had died during infancy but were themselves found to be healthy. The remaining 8 samples were collected in the obstetrical department from infants born after normal pregnancies and deliveries. In addition three infants in the recovery period after non-surgical treatment for iliac stenosis were also investigated.

Hyperbilirubinemic infants

Because of the difficulties in obtaining from normal infants the large amount of plasma needed for the bioassays hyperbilirubinemic infants with normal hemoglobin levels were also investigated. Twenty one of these infants were 2 and 3 days old. The cause of the hyperbilirubinemia in these infants was ABO incompatibility. Four of these infants were premature. Hemoglobin levels were above 18.9 g/ml and total bilirubin between 13 and 23 mg/100 ml. The reticulocyte levels were within normal range. Coombs test was negative. None of these infants were readmitted later because of prolonged jaundice.

The clinical data on the 25 hyperbilirubinemic infants 4 to 6 days old are listed in Tables 2-4. These infants had hemoglobin levels above 11.2 g/100 ml and reticulocyte levels below 2.5% at the time of blood sampling. The Coombs test was negative in all infants. In 10 infants the percentage of unconjugated bilirubin was in the range of 83-95%. Blood was collected during exchange transfusion. In premature infants the first 20 ml of blood withdrawn was sampled and in mature infants the first 40 ml.

Controls

Plasma from normal adults with normal hemoglobin was used for control samples. To test the possibility that unconjugated bilirubin was influencing the erythropoiesis in the recipient mice bilirubin was added to the normal adult plasma in the following manner: To 5 mmol EDTA + 0.4% NaHCO₃ was added 0.1 M NaOH until pH reached 11.40 mg bilirubin (Sigma) was added and mixed with citrated normal

Table 1 *Effect of plasma from normal newborn infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours*

Relevant clinical and laboratory data are listed. The ^{59}Fe uptake values in all recipient mice in each group are pooled and the mean and S.E.M. are shown. The statistical significance of the difference between the plasma treated group and the saline controls is indicated by asterisks in this and subsequent tables (***- $p < 0.001$ **- $0.001 < p < 0.005$ *- $0.005 < p < 0.01$). Figures in parentheses indicate number of recipient mice

Name	Age (days)	Birth weight/length (g/cm)	Hemoglobin (g/100 ml)	Reticulocytes ()	Percent ^{59}Fe uptake in RBC of recipient mice \pm S.E
G U A	4	3 250/51	20.7	1.0	8.2 \pm 0.3 (2) ***
G U N	4	2 930/49	21.0	0.8	8.8 \pm 1.2 (4) ***
A N D	4	2 780/49	21.0	1.1	9.5 \pm 0.9 (2) ***
K A M	5	2 520/48	20.7	1.7	8.8 \pm 0.1 (5) ***
B J E	6	3 120/51	20.8	0.4	7.9 \pm 1.1 (4) ***
Neonatal plasma pooled					8.6 \pm 0.4 (17) ***
Saline pooled					33.7 \pm 2.6 (26) —
Adult plasma pooled					26.1 \pm 2.0 (31) n.s.

adult plasma which was used in the exchange transfusion and pH was adjusted to 7.5 with 0.1 N HCl (8). The bilirubin concentration in the plasma was determined according to Jendrasik & Grof (11). In one assay different fractions from an exchange transfusion were used to test the effect of dilution. The first sample consisted of the first 40 ml of infant blood, the second fraction the next 40 ml and the third fraction the third 40 ml blood drawn from a hyperbilirubinemic 3 day old infant.

BIOASSAY

Randomized female mice of the NMRI/BOM strain weighing between 25 and 30 g were used. The mice were made polycythemic by hypoxia in a low pressure chamber at 0.4 atm for 3 weeks. Following removal from the chamber the erythropoiesis decreased to zero. On the fourth day out of chamber erythropoiesis was again stimulated by endogenous or exogenous erythropoietin. Endogenous erythropoietin was produced by 6–11 hours of hypoxia (0.4 atm). The plasma to be tested and saline were given immediately before and after this second hypoxic period and twice on the following day. As exogenous erythropoietin was used an internal laboratory standard of erythropoietin obtained by dialysis and lyophilization of urine from a patient with aplastic anemia. The erythropoietin standard was injected subcutaneously and simultaneously with the plasma to be tested or saline in doses of 0.1 mg at 9 a.m. and 4 p.m. on the fourth day and 0.2 mg at the same hours on the fifth day out of tank. Altogether 0.6 mg of the internal standard was injected and this is equal to 0.15 units of the Standard Erythropoietin II in our assay. The mice responded to these doses with approximately 10% ^{59}Fe uptake in RBC. In some assays a total dose of either 0.08 or 4.0 mg was injected (Table 4). In all experiments the mice received 0.5 ml \times 4 of untreated plasma or 0.4 ml \times 4 of

saline. 0.1 μCi ^{59}Fe dissolved in 0.5 ml saline was injected intraperitoneally at 9 a.m. on the sixth ex-hypoxic day and the mice were venipunctured by the femoral vein after ether anaesthesia 48 hours later. A well scintillation counter was used (Frieske & Hoepfner Erlangen Bruch Germany). The radioactivity in 0.5 ml blood sample was measured with a gamma sensitive crystal with an efficiency of about 6.5×10^4 cpm per 0.1 μCi of ^{59}Fe at a background of 440–470 cpm. Mice with a hematocrit lower than 53 were excluded. The inhibition was calculated from the difference in ^{59}Fe uptake between the control mice given saline and the groups of mice given plasma injections.

RESULTS

Table 1 shows the effect of normal neonatal plasma on ^{59}Fe incorporation in the red blood cells (RBC) of plethoric mice stimulated with hypoxia. Plasma withdrawn from normal newborns between the 4th and 6th days significantly reduced the ^{59}Fe incorporation in the red blood cells of the recipient mice compared with saline controls.

Table 2 and 3 show the effect of plasma sampled during exchange transfusions performed on the 4th, 5th and 6th days of life.

Table 2 depicts the results in mice in which the erythropoiesis was stimulated with 8 hours hypoxia and Table 3 in mice in which erythropoiesis was stimulated with injections of 0.6 mg of our internal erythropoietin standard. In both types of recipients the ^{59}Fe incorporation

Table 2 *Effect of plasma from newborn hyperbilirubinemic infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours*

Relevant clinical and laboratory data are listed. The ^{59}Fe uptake in all recipient mice in each group are pooled and the mean and S.E.M. are shown

Name	Age (days)	Birth weight/length (g/cm)	Clinical comments	Hemoglobin (g/100 ml)	Reticulo-cytes (%)	Bilirubin (mg/100 ml)	Percent ^{59}Fe uptake in RBC of recipient mice \pm S.E.
L.H.S.	4	1 600/40	Premature	18.2	1.2	17.4	3.6 ± 0.4 (8) *
Y.P.E.	4	1 800/41	Premature	20.9	0.5	22.2	3.5 ± 0.4 (5) ***
K.W.E.	4	2 890/48		19.4	2.5	25.0	6.2 ± 0.8 (5)
E.L.A.	4	3 640/49	Diabetic mother	18.2	1.3	23.0	3.5 ± 0.8 (7) *
M.S.K.	5	3 680/50		19.5	1.3	35.0	6.4 ± 0.7 (6) **
N.E.A.	6	1 960/44	Premature	18.8	2.0	19.7	5.4 ± 1.1 (7)
Neonatal plasma pooled							4.7 ± 0.5 (38) **
Saline pooled							12.1 ± 1.2 (33) —
Adult plasma pooled							15.4 ± 1.9 (28) n.s.

was significantly reduced when the animals were injected with plasma from newborn infants aged 4–6 days compared with mice injected with saline or normal adult plasma.

Table 4 illustrates the clinical and laboratory data of the 4–6-day-old infants not presented in Tables 1–3. The degree of the second erythropoietic stimulation is different from that in the assays presented in the previous tables.

Table 5 shows the effect of bilirubin on erythropoiesis. Plasma with added bilirubin increased the ^{59}Fe uptake however not significantly while the neonatal plasma with the

same bilirubin content significantly reduced the uptake compared with the saline group.

Plasma withdrawn between the age of 4–6 days of life from premature and mature infants from normal and hyperbilirubinemic infants significantly inhibited the ^{59}Fe incorporation in the RBC of the recipient mice. Since there is good agreement between these results it has been found justified to visualize all the data in one figure. In Fig. 1 is included the data from all the assays performed with plasma from infants irrespective of the clinical condition of the donor infant and the type of sec-

Table 3 *Effect of plasma from newborn hyperbilirubinemic infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with 0.6 mg of an internal laboratory standard of erythropoietin*

Relevant clinical and laboratory data are listed. The ^{59}Fe uptake in all recipient mice in each group are pooled and the mean and S.E.M. are shown

Name	Age (days)	Birth weight/length (g/cm)	Clinical comments	Hemoglobin (g/100 ml)	Reticulo-cytes (%)	Bilirubin (mg/100 ml)	Percent ^{59}Fe uptake in RBC of recipient mice \pm S.E.
U.G.R.	4	3 210/47	ABO incompatible	20.8	1.3	21.6	2.0 ± 0.2 (5)
J.E.A.	4	2 300/47	Premature	20.9	1.4	23.0	1.7 ± 0.3 (6) *
E.L.A.	4	3 640/49	Diabetic mother	18.2	1.3	23.0	2.7 ± 0.5 (5) ***
T.V.E.	5	3 430/49		20.9	1.2	29.5	2.0 ± 0.5 (5)
P.L.Y.	6	1 960/43	Premature	20.9	1.2	21.0	2.2 ± 0.2 (5)
S.C.H.	6	3 025/50		21.7	1.7	21.0	2.1 ± 0.2 (6) ***
Neonatal plasma pooled							2.0 ± 0.2 (33)
Saline pooled							9.6 ± 0.7 (23)
Adult plasma pooled							9.3 ± 1.2 (20)

Table 1 Effect of plasma from normal newborn infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours

Relevant clinical and laboratory data are listed. The ^{59}Fe uptake values in all recipient mice in each group are pooled and the mean and S.E.M. are shown. The statistical significance of the difference between the plasma treated group and the saline controls is indicated by asterisks in this and subsequent tables (*** = $p < 0.001$, ** = $0.001 < p < 0.005$, * = $0.005 < p < 0.01$). Figures in parentheses indicate number of recipient mice.

Name	Age (days)	Birth weight/length (g/cm)	Hemoglobin (g/100 ml)	Reticulocytes (%)	Percent ^{59}Fe uptake in RBC of recipient mice \pm S.E.
G U A	4	3 250/51	20.7	1.0	8.2 \pm 0.3 (2) ***
G U N	4	2 930/49	21.0	0.8	8.8 \pm 1.2 (4) ***
A N D	4	2 780/49	21.0	1.1	9.5 \pm 0.9 (2) ***
A A M	5	2 520/48	20.7	1.7	8.8 \pm 0.1 (5) ***
B J E	6	3 120/51	20.8	0.4	7.9 \pm 1.1 (4) ***
Neonatal plasma pooled					8.6 \pm 0.4 (17) ***
Saline pooled					33.7 \pm 2.6 (26) —
Adult plasma pooled					26.1 \pm 2.0 (31) ns

adult plasma which was used in the exchange transfusion and pH was adjusted to 7.5 with 0.1 N HCl (8). The bilirubin concentration in the plasma was determined according to Jendrasik & Gros (11). In one assay different fractions from an exchange transfusion were used to test the effect of dilution. The first sample consisted of the first 40 ml of infant blood, the second fraction the next 40 ml and the third fraction the third 40 ml blood drawn from a hyperbilirubinemic 3 day old infant.

BIOASSAY

Randomized female mice of the NMRI/BOM strain weighing between 25 and 30 g were used. The mice were made polycythemic by hypoxia in a low pressure chamber at 0.4 atm for 3 weeks. Following removal from the chamber the erythropoiesis decreased to zero. On the fourth day out of chamber erythropoiesis was again stimulated by endogenous or exogenous erythropoietin. Endogenous erythropoietin was produced by 6–11 hours of hypoxia (0.4 atm). The plasma to be tested and saline were given immediately before and after this second hypoxic period and twice on the following day. As exogenous erythropoietin was used an internal laboratory standard of erythropoietin obtained by dialysis and lyophilization of urine from a patient with aplastic anemia. The erythropoietin standard was injected subcutaneously and simultaneously with the plasma to be tested or saline in doses of 0.1 mg at 9 a.m. and 4 p.m. on the fourth day and 0.2 mg at the same hours on the fifth day out of tank. Altogether 0.6 mg of the internal standard was injected and this is equal to 115 units of the Standard Erythropoietin B in our assay. The mice responded to these doses with approximately 10% ^{59}Fe uptake in RBC. In some assays a total dose of either 0.08 or 4.0 mg was injected (Table 4). In all experiments the mice received 0.5 ml \times 4 of untreated plasma or 0.4 ml \times 4 of

saline. 0.1 μCi ^{59}Fe dissolved in 0.5 ml saline was injected intraperitoneally at 9 a.m. on the sixth ex hypoxic day and the mice were venipunctured by the femoral vein after either anesthesia 48 hours later. A well scintillation counter was used (Frisseke & Hoepfner Erlangen Bruch Germany). The radioactivity in 0.5 ml blood sample was measured with a gamma sensitive crystal with an efficiency of about 6.5×10^4 cpm per 0.1 μCi of ^{59}Fe at a background of 440–470 cpm. Mice with a hematocrit lower than 53 were excluded. The inhibition was calculated from the difference in ^{59}Fe uptake between the control mice given saline and the groups of mice given plasma injections.

RESULTS

Table 1 shows the effect of normal neonatal plasma on ^{59}Fe incorporation in the red blood cells (RBC) of plethoric mice stimulated with hypoxia. Plasma withdrawn from normal newborns between the 4th and 6th days significantly reduced the ^{59}Fe incorporation in the red blood cells of the recipient mice compared with saline controls.

Table 2 and 3 show the effect of plasma sampled during exchange transfusions performed on the 4th, 5th and 6th days of life.

Table 2 depicts the results in mice in which the erythropoiesis was stimulated with 8 hours hypoxia and Table 3 in mice in which erythropoiesis was stimulated with injections of 0.6 mg of our internal erythropoietin standard. In both types of recipients the ^{59}Fe incorporation

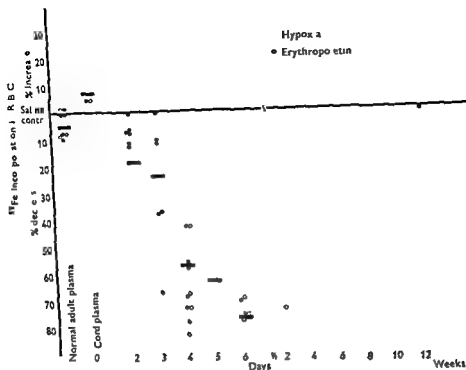


Fig. 1 Effect of neonatal plasma on ^{59}Fe uptake in RBC of polycythemic mice in which erythropoiesis has been stimulated with endogenous (hypoxia) or exogenous erythropoietin. The baseline illustrates ^{59}Fe uptake levels of saline injected mice. Circles or points

represent the mean of each plasma injected group expressed as percentage increase or decrease of ^{59}Fe uptake in relation to saline control levels. — Mean of pooled data in each type of plasma. Age of infants at time of sampling is indicated at the baseline.

poiesis was stimulated by hypoxia or exogenous erythropoietin. The neonatal plasma also inhibited the reticulocyte response to hypoxia in mice. The latter data will be reported elsewhere (25).

The results are in contrast with those of Matoth & Zaizov (16). These authors could not demonstrate any inhibitory effect of pooled plasma withdrawn from 6-day-old infants on the ^{59}Fe uptake in red blood cells of normal mice which were stimulated by 18 hours hypoxia. Neither did neonatal plasma inhibit the erythropoietic effect of plasma from an anemic thalassemia patient, using hypertransfused mice as recipient animals (16).

Several methods for determination of erythropoiesis inhibiting factors have been described and are discussed elsewhere (25–28). The present authors are of the opinion that the post hypoxic polycythemic mice are the most sensi-

tive recipient animals in erythropoiesis inhibition studies. From our data it also seems important that the inhibiting factor is injected into the recipient animals shortly before or at the same time as the stimulator of erythropoiesis is applied. The differences between our results and the results of Matoth & Zaizov may possibly be explained by the differences in the assay systems.

Because of the restricted amount of blood that can be obtained from healthy newborn infants plasma was also drawn from hyperbilirubinemic infants during exchange transfusions. Both normal and jaundiced infants had normal hemoglobin levels and showed evidence of the normal suppression of erythropoiesis after birth. A low percentage of conjugated bilirubin indicated that the cause of the hyperbilirubinemia was immature liver function and not excessive hemolysis. Bilirubin has previ-

Table 4 Relevant clinical and laboratory data of infants between 4 and 84 days of age

The percentage inhibition of ^{59}Fe incorporation into RBC of hypoxia induced polycythemic mice stimulated with hypoxia or with an internal laboratory standard of erythropoietin is indicated. The mean of the percentage of inhibition and S.E.M. for each infant group are given. The statistical significance of the difference between infant plasma injected mice and saline injected mice is indicated by asterisks (see Table 1).

Name	Age (days)	Birth weight/length (g/cm)	Clinical comments	Hemo-globin (g/100 ml)	Reti-culo-cytes ()	Bili-rubin (mg/100 ml)	Content (un conjug)	Percentage inhibition \pm S.E. of ^{59}Fe uptake	Injected mice n
C L A	4	2 320/46	Premature	18.0	11	18.2	94	36 \pm 2.7	6 **
P A F	4	1 540/41	Prem ABO incomp	20.8	19	21.4	90	22 \pm 3.8	5 *
E N O	4	3 350/52	ABO incompatibility	19.3	0.4	21.4	83	73 \pm 4.4	6 ***
R U D	4	1 800/42	Diabetic mother	18.8	2.0	20.5	—	42 \pm 5.2	4 **
S K O	4	3 495/41	Hyperbilir	18.8	2.0	18.8	95	83 \pm 5.5	6 ***
T R L	5	3 100/49	AO incomp	19.3	2.0	22.6	—	55 \pm 4.0	6 ***
T T H	5	1 738/38	AO incomp prem	20.8	1.5	25.0	89	56 \pm 6.0	8 ***
H M N	6	1 830/44	Prem	20.8	0.4	18.7	88	76 \pm 4.6	8 ***
L I S	5	3 800/52	BO incomp	18.8	1.2	11.0	—	74 \pm 3.2	4 ***
G A A	5	3 185/52	Hyperbilir forceps	18.0	2.0	13.9	—	83 \pm 6.1	4 ***
H O F	13	3 250/51	Normal	18.8	1.0	1.3	—	72 \pm 4.4	4 ***
S Y F	14	3 600/53	Normal	16.1	0.6	—	—	68 \pm 12.0	5 **
N A N	28	2 940/50	Normal	16.5	0.5	—	—	38 \pm 14.2	4 *
K O R	50	3 600/53	Pyloric stenosis	13.2	0.7	—	—	40 \pm 9.2	5 **
G O L	56	3 980/51	Pyloric stenosis	13.5	0.6	—	—	22 \pm 10.1	5 n.s.
S K U	70	3 200/51	Pyloric stenosis	12.6	2.1	—	—	34 \pm 9.0	5 *
S T E	84	3 850/53	Normal	12.0	4.2	—	—	3 \pm 4.0	5 n.s.

ondary stimulation of erythropoiesis in the recipient mice. Fig. 1 shows that plasma from normal adults reduced the ^{59}Fe uptake slightly but insignificantly in the recipient mice. Mice injected with plasma from cord blood had a higher ^{59}Fe uptake than the mice injected with saline, but the difference was not significant. Mice injected with plasma withdrawn from infants aged 2 days to 6 weeks had a lower uptake than the controls, and the differences were

Table 5 Effect of bilirubin added to normal adult plasma on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 6 hours

The bilirubin content of the injected material is given

Injected material	Total bilirubin content (mg/100 ml)	Percent ^{59}Fe uptake in RBC of recipient mice \pm S.E.
Adult plasma	1.2	72 \pm 0.8 (5) n.s.
Adult plasma with added bilirubin	25.0	113 \pm 1.3 (5) n.s.
Neonatal plasma	22.2	35 \pm 0.4 (5) ***
Saline	—	78 \pm 0.3 (8) —

significant from the 4th to the 14th day of life. From the age of 6–12 weeks no significant inhibitory effect of the plasma could any longer be demonstrated. Plasma obtained at the 2nd and 3rd days of life inhibited the ^{59}Fe uptake, however insignificantly.

When plasma from a 3 day old infant withdrawn during an exchange transfusion was separated in portions of 40 ml, one could demonstrate a diluting effect on the inhibitory influence. Fig. 2 illustrates the ^{59}Fe uptake in the recipient mice after injections with the different fractions compared with the effect of the donor plasma in the actual transfusion and with saline. Plasma from the first 40 ml and the second 40 ml had a significant inhibitory effect on the ^{59}Fe uptake of the red blood cells in the polycythemic mice stimulated with endogenous erythropoietin.

DISCUSSION

Plasma from normal and hyperbilirubinemic newborn infants inhibited ^{59}Fe incorporation in RBC of polycythemic mice in which erythro-

and 12 weeks of age did not consistently show any inhibitory effect. In this age group the hemoglobin concentration falls to its lowest level after birth. Halvorsen (9) was unable to demonstrate any erythropoietin in plasma from infants below 54 days of life but the assay is not sensitive enough to differentiate between normal and low normal erythropoietin levels. At present one cannot therefore state whether the reduction of inhibitory activity is due to disappearance of the inhibitor or an increase in plasma erythropoietin which counteracts the inhibitor.

The physiological role of inhibitors in erythropoiesis is still debated (3, 16) and it is so far unknown whether the inhibitors are constantly present in plasma or urine or produced as a response to a surplus of oxygen available in the tissues. The present data do not provide evidence in any of these directions but studies on urine samples after separation of erythropoietin from the inhibitor may give the answer to this question (15).

The cellular growth in epidermis is regulated by tissue specific inhibitors of mitosis termed chalones by Bullock (2). Rytomaa & Lavi-niemi (22) have extracted inhibitors of myelopoiesis and erythropoiesis from mature granulocytes and erythrocytes and they have also been able to demonstrate such substances in serum. The relationship between the inhibitors in plasma of newborn infants and the erythrocytic chalone can only be speculated upon at the present time. However it is reasonable to suggest that the erythropoietic inhibitors may be related to chalones and that they are of physiological importance. In the neonatal period such inhibitors may effectively depress erythropoiesis and the hematological changes following birth may be explained as a result of variations in the stimulators and inhibitors of erythropoiesis.

SUMMARY

The marked and rapid decrease of erythropoiesis in the normal newborn infants may be due to disappearance of erythropoietin alone but

an inhibitory mechanism may also be involved. Several authors have previously reported the presence of erythropoiesis inhibiting factors in plasma from high altitude natives brought to sea level and in plasma from hypertransfused animals. The present studies of ^{59}Fe incorporation in RBC of polycythemic mice injected with plasma from normal and hyperbilirubinemic newborn infants show that erythropoietic inhibitor(s) is present in the plasma of infants at least from the fourth day of life. This inhibition was not due to the bilirubin content. A possible influence of estrogen was discussed and found to be unlikely. The relation to chalones, the tissue specific inhibitors of mitosis is discussed.

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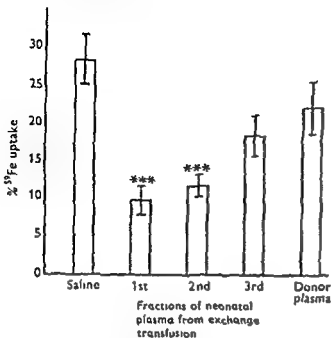


Fig 2 Effect of plasma from a 3 day old hyperbilirubinemic infant withdrawn during the first part of an exchange transfusion on ^{59}Fe uptake in RBC of polycythemic mice in which erythropoiesis has been stimulated with endogenous erythropoietin (hypoxia). The statistical significance of the difference between the plasma injected group and saline controls is indicated by asterisks (*** - $p < 0.001$). Columns represent the mean of each assay group \pm SE.

ously been reported to have an erythropoiesis stimulating effect (1). In our assay there was no statistically significant difference in the ^{59}Fe uptake in RBC of mice injected with normal adult plasma, with or without added bilirubin.

The reduced ^{59}Fe uptake in the RBC of the recipient mice following plasma injections may be caused by different mechanisms. A reduced iron incorporation into heme or a specific inhibition of the erythropoietic stimulating effect of erythropoietin are the two most likely mechanisms.

Based on analysis of serum iron in the injected plasmas and simultaneous ^{59}Fe uptake and reticulocyte studies in the recipient mice (25), the present authors favour the interpretation that the reduction in ^{59}Fe uptake is due to specific inhibitors of erythropoiesis. This is in accordance with the interpretations of the findings of proteins which reduce ^{59}Fe uptake in recipient animals (7, 14, 15, 19). At which

cellular level this inhibition occurs, is, however, unclear.

An inhibitory effect of estrogens on erythropoiesis has been demonstrated previously (17). Since the estrogen content in newborn plasma is reported to be high (18) the inhibitory effect could be explained by high estrogen levels. The level is, however, higher in cord plasma than later in the first week, which does not fit with our findings that the inhibition is most marked from the fourth day of life (18). The doses of estrogen used in the inhibition studies were much higher than the possible content in 2 ml infant plasma. It is therefore unlikely that the inhibitory effect in our experiments could be an estrogen effect.

The second question to be commented upon is the time of appearance of the inhibitor in infant plasma after birth. In plasma from high altitude natives brought to sea level, Reynafarje could demonstrate an inhibitory effect on ^{59}Fe uptake after 72 hours and an optimal effect on the 7th-9th days after transfer to sea level (21). Whitcomb & Moore reported that the inhibitory effect of sheep plasma could not consistently be demonstrated until 72 hours following hypertransfusion (28).

In our present experiments plasma drawn from 2 day-old infants exhibited an inhibitory effect on the ^{59}Fe uptake. The pooled means, however, were not statistically significant in infants below 4 days of age. Plasma from older infants caused a 70% reduction of iron uptake compared to the saline controls. Halvorsen and Finne have previously demonstrated high levels of erythropoietin in cord blood (6, 9). These findings are consistent with the concept that the fetus produces erythropoietin as a response to hypoxia (5, 6, 24). Following birth the erythropoietin levels decrease to not detectable levels (9) and it is assumed that the erythropoietin production stops. The lag period before erythropoietic inhibitors are demonstrable in plasma may be the result of the time necessary for erythropoietin to disappear from plasma.

Plasma withdrawn from infants between 6

FAILURE TO THRIVE IN LEBANON I EXPERIENCE WITH SOME SIMPLE SOMATIC MEASUREMENTS

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Some years ago an exploratory study of the socio-economic background of marasmus in Lebanon was undertaken by following over the course of 1 year nearly 200 consecutive cases after discharge from hospital (19). By the end of this period about 15 from the city of Beirut and 30 from the south of Lebanon had died and of the survivors more than 50% were below the 3rd percentile (Boston) for weight. Since then we have investigated many aspects of the problem of protein-calorie malnutrition (P.C.M.) and some of our findings have been summarised recently (22). Experience in Jordan and Lebanon led to the development of a concept of the evolution of marasmus and kwashiorkor under different ecologic conditions (23) and a simple Scoring System by which to classify the various forms of severe P.C.M. was devised (24). This has been applied over a period of 12 months in a hospital in Amman, Jordan together with the collection of data on ecologic factors that might be related to undernutrition (18, 25).

At the same time we have been studying the nature of the "submerged" subclinical part of the P.C.M. iceberg from which arise the severe cases seen in hospital. Several studies with a rather similar objective have been published from other countries (1-4, 10, 12, 13, 20, 26-28, 30, 33). Although circumstances vary considerably from one place to another

and care must be exercised when comparisons are made we believe that our results are of interest to those concerned with the health of the pre school child in other developing countries. Our primary intention in this paper has been to devise and test simple tools for the study of failure to thrive in the field rather than to investigate a representative population sample and obtain data on prevalence and incidence. In a later paper we intend to present the results we have obtained when the method devised and described here was applied in the field.

MATERIAL AND METHODS

The subjects in this study were Arab children between the ages of 3 and 48 months of Moslem Shii or Sunni families who attended a clinic in one of the three areas of Lebanon. These areas were 1) the village of Jbaa, in the south of the country 2) Bourj al Barajne a suburb of Beirut and 3) Basta, an area in downtown Beirut. The groups for study were chosen serially from those children who were attending the clinic for immunization or for trivial illness. Those with serious illnesses were excluded. The entire study was carried out by the same Arab physician (A. K.) assisted by the same Arab public health nurse (I. A. J.) and took 12 months to complete (December 1967 to November 1968).

The data collected at this stage of the investigation consisted of name, address, date of birth, certain somatic measurements and clinical signs. Only children with identity cards were included. Most children had these but they were not necessarily reliable. A calendar was devised using events of the

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MATERIAL AND METHODS

The subjects in this study were Arab children between the ages of 3 and 48 months of Moslem Shii or Sunni families who attended a clinic in one of the three areas of Lebanon. These areas were 1) the village of Jba'a in the south of the country, 2) Bourj al Barajne a suburb of Beirut and 3) Basta an area in downtown Beirut. The groups for study were chosen serially from those children who were attending the clinic for immunization or for trivial illness. Those with serious illnesses were excluded. The entire study was carried out by the same Arab physician (A. K.) assisted by the same Arab public health nurse (I. A. J.) and took 12 months to complete (December 1967 to November 1968).

The data collected at this stage of the investigation consisted of name, address, date of birth, certain somatic measurements and clinical signs. Only children with identity cards were included. Most children had these but they were not necessarily reliable. A calendar was devised using events of the

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Table 3 Four somatic measurements expressed as percentages of standards according to season of observation

	standards (mean \pm S D)			
	Spring 239 cases	Summer 242 cases	Autumn 283 cases	Winter 467 cases
Weight	91.0 \pm 13.4	89.0 \pm 12.8	91.0 \pm 14.5	91.0 \pm 13.7
Height ^a	97.1 \pm 4.8	97.6 \pm 5.0	98.0 \pm 5.0	97.1 \pm 5.7
Head circumference	96.5 \pm 4.6	96.2 \pm 3.6	96.2 \pm 3.3	96.1 \pm 3.4
Mid arm circumference	92.0 \pm 4.3	89.9 \pm 8.1	89.6 \pm 8.6	89.9 \pm 8.4

^a 0.02 > *p* > 0.01 Summer v rest of year^b 0.05 > *p* > 0.02 Autumn v rest of year^c *p* < 0.001 Spring v rest of year

and mid arm circumference did not differ significantly in the two sexes

Table 2 shows the same data divided according to location. Only for height were there significant differences between the three areas.

In Lebanon the year may be divided from the climatic point of view into four rather distinct seasons, each of 3 months duration: spring, March–May; summer, June–August; autumn, September–November; and winter, December–February. Table 3 shows the data compared with regard to the season when they were collected. Weight was significantly less in summer than in each of the other seasons; height was significantly greater in autumn than in any of the other seasons; head circumference differed not according to season; and mid arm circumference was highly significantly greater in spring than in any of the other seasons.

In Table 4 a similar comparison is made for season of birth. In winter weight was significantly less than in spring and height less than in the other seasons.

Table 4 Four somatic measurements expressed as percentages of standards according to season of birth

	standards (mean \pm S D)			
	Spring 94 cases	Summer 161 cases	Autumn 268 cases	Winter 310 cases
Weight	92.0 \pm 13.5	90.6 \pm 18.5	90.5 \pm 14.9	89.3 \pm 12.1
Height ^a	97.6 \pm 5.1	97.9 \pm 5.2	98.0 \pm 5.7	96.9 \pm 4.8
Head circumference	96.4 \pm 5.4	96.4 \pm 3.8	96.3 \pm 3.7	96.2 \pm 3.6
Mid arm circumference	91.0 \pm 8.1	89.7 \pm 8.5	89.5 \pm 8.7	90.7 \pm 7.9

^a 0.01 > *p* > 0.001 Winter v spring.^b 0.02 > *p* > 0.01 Winter v rest of year

The correlations between the four somatic measurements are shown in Table 5. The correlations are much higher for the actual values of the measurements than the measurements expressed as percentage of standard.

The four somatic measurements were used to classify the subjects according to the growth they had achieved in comparison with the international standards. Several systems were applied. The results obtained were almost identical but certain advantages were gained with each modification. Initially an Index of Thriving employing all four somatic measurements in relation to international standards was used (17). This is quite satisfactory as a research tool but for widespread routine use it has the disadvantage of requiring a weighing machine, an expensive delicate instrument often not used properly by semi-skilled personnel. The first refinement consisted of the head circumference and the mid arm circumference in relation to international standards. The measurements are simple to make and require

Table 1 Four somatic measurements expressed as percentage of standards, in males and females

	standards (mean \pm S D)		p
	Males 636 cases	Females 595 cases	
Weight	93.4 (\pm 13.5)	87.6 (\pm 13.2)	<0.001
Height	98.0 (\pm 5.0)	96.8 (\pm 5.9)	<0.001
Head circumference	96.2 (\pm 3.6)	96.2 (\pm 3.7)	NS ^a
Mid arm circumference	90.5 (\pm 8.1)	89.9 (\pm 8.6)	NS

^a NS = Not significant

Moslem year and local occurrences to check the age of the child to the nearest month. Four somatic measurements: weight, height, head circumference and mid arm circumference were made according to the procedure recommended by WHO (14). The presence or absence of oedema, hepatomegaly, dermatosis and hair changes of protein-calorie malnutrition according to the descriptions of WHO (32) were noted.

RESULTS

The sample comprised 1231 children, 636 males and 595 females. The largest number was seen in Bourj al Barajne (541) where the most active clinic is to be found. There were 386 in Basta and 304 in Jba'a. The proportional distribution by sex and age was similar in the three locations. It was therefore considered in order to pool data from all three locations for certain comparisons.

Figs 1 and 2 show for males and females separately, mean values for the four somatic measurements expressed in relation to international standards (Boston standards for weight, height, and head circumference (29) and Warsaw (31) for mid arm circumference).

The curves for the four measurements were similar for the two sexes but there were some differences. For boys and girls height and head circumference tended to be close together and relatively less affected than weight and mid arm circumference which also tended to remain close together. There was a steady falling off in head circumference in both sexes from the age of 3 months with the value being ap-

proximately 95% Boston for most of the period under study. The height in both sexes tended to be nearer to the Boston standard than the head circumference during the first 12 months and for much of this time was equivalent to 100% Boston. In the second year it fell below 95% Boston and during the 3rd and 4th years showed a greater deficit in both sexes than did head circumference.

Weight in the male was nearly 100% Boston at 3 months and at or above this level until 6 months, after which it fell drastically to about 85% by 12 months. The same downward trend occurred in girls but at a lower level. In both sexes there was thereafter a slow and fairly steady increase of weight percentage until 4 years. The mid arm circumference in the female followed the weight rather closely in the first year. In the male it reached the same low level then but without any early rise as was the case for weight. During the 2nd to 4th years there was a steady increase in mid arm circumference in both sexes.

The data for the four somatic measurements, expressed as percentages of the international standards, are compared for males and females of the entire sample in Table 1. For both sexes weight and mid arm circumference fell further short of the standards than did height and head circumference. The percentage weight and height of girls were highly significantly lower than those of boys. The head circumference

Table 2 Four somatic measurements expressed as percentages of standards in three locations

	standards (mean \pm S D)		
	Basta 386 cases	Bourj al Barajne 541 cases	Jba'a 304 cases
Weight	90.1 (\pm 13.9)	91.1 (\pm 13.9)	90.0 (\pm 13.0)
Height ^a	98.0 (\pm 5.1)	97.3 (\pm 5.0)	96.9 (\pm 5.4)
Head circumference	96.0 (\pm 3.4)	96.3 (\pm 4.1)	96.2 (\pm 3.2)
Mid arm circumference	90.1 (\pm 7.2)	90.2 (\pm 8.3)	90.5 (\pm 8.2)

^a 0.02 > p > 0.01 Basta v Jba'a

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Table 3 Four somatic measurements expressed as percentages of standards according to season of observation

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Mid arm circumference	92.0 \pm 4.3	89.9 \pm 8.1	89.6 \pm 8.6	89.9 \pm 8.4

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^a 0.01 > *p* > 0.001 Winter v spring.^b 0.01 > *p* > 0.01 Winter v rest of year.

Table 5 Correlation coefficients (*r*) for four somatic measurements expressed as actual values and as percentages of standards

	<i>r</i> using actual values	<i>r</i> using percentages of standards
Weight v Height	0.943	0.671
Weight v Head circum ference	0.857	0.558
Weight v Mid arm circum ference	0.787	0.717
Height v Head circum ference	0.843	0.445
Height v Mid arm circum ference	0.650	0.305
Head circumference v Mid arm circumference	0.659	0.408

only a stout tape measure. However, age needs to be known. It was subsequently found that the ratio of these two measurements between 3 months and 4 years is constant for healthy children of both sexes. It correlates highly with the Index of Thriving (19).

Thus, by any one of several means it is possible to compare the growth of pre-school children. Using the Index of Thriving two contrasting groups were identified: those growing well as a control group, and those growing poorly as a failure to thrive group. Detailed studies of environmental factors operating in these two groups and a long-term follow-up and evaluation of the methods used form the substance of subsequent reports.

DISCUSSION

Assessment of failure to thrive

The need has long been recognized for a simple, objective means which can be used by general public health workers with a limited knowledge of nutrition to assess the physical development of population groups, especially pre-school children, in relation to their nutritional status.

Gomez et al. (6) first advocated the division of malnutrition (P.C.M.) into 1st, 2nd and 3rd degrees according to the percentage deviation of the weight from the mean of a local

standard group. This classification has been widely adopted and has proved of great value in enabling results to be compared from different places. However, standards established under differing circumstances of place, time, methodology and population examined are bound to differ. Reference to a widely recognized international standard will assist in bringing uniformity of comparison but it assumes that healthy child populations everywhere measure the same. This is not true if inborn effects of retardation in previous generations are present.

There is evidence that healthy Lebanese children of a high socio-economic class have weights and heights similar to those of European children (11). For routine use under field conditions, weight has certain disadvantages. A weighing machine is a delicate instrument, requiring intelligent use for reliable results. The age must be known to the nearest month or so.

For research purposes it seemed to us desirable to employ several measurements. Body weight includes all compartments of the body and gives no indication of disproportionate changes in the size of different compartments. Continuing increase in weight may bring a false sense of security when accumulation of water is taking place in early protein deficiency. Conversely, considerable falling off in weight gain velocity or actual loss of weight may occur over a short period of time in association with a brief but severe illness. Height indicates the combined length of certain parts of the skeleton, a part of the body relatively little affected by malnutrition. Height cannot decrease absolutely. Head circumference is made up mainly of external skull circumference but also includes small contributions from skin, subcutaneous tissue and muscle. It correlates closely with brain size and at the lowest ranges with mental function. Being mainly a skeletal measurement it behaves like height. Mid arm circumference includes bone, muscle, fat, subcutaneous tissue and skin. As might be expected from its multi-tissue nature, it behaves much like body weight. Other somatic meas-

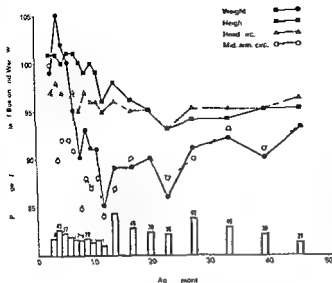


Fig 1 Four somatic measurements expressed as percentages of standards (see text) in relation to age. Number in each age group at foot of graph. Data for boys.

measurements some singly and others as ratios have been proposed but these also have various disadvantages including their age-dependence (16). Certain laboratory tests involve the additional difficulties of collection of samples and their processing.

We have studied each of these approaches to the problem and as indicated in the results presented here have come by way of the use of four somatic measurements in the Index of Thriving finally to select two of these for

routine field use. They are mid arm circumference and head circumference expressed as a ratio which as we have shown elsewhere (19) appears to have none of the above mentioned disadvantages.

Growth pattern of pre school children

The growth pattern as judged by the four somatic measurements shown in Figs 1 and 2 approximates to that of children in many developing countries (2, 4, 10, 12-14, 20, 26).

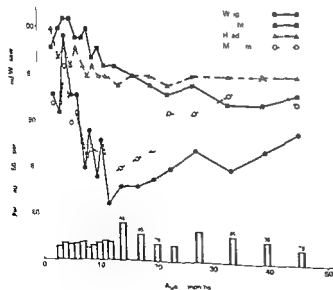


Fig 2 As Fig 1. Data for girls.

27, 29, 33) The gains in height and weight are satisfactory for the first 3-6 months of life. After the sixth month weight falls to about 85% by the 12th month. Similar declines were reported but to differing degrees from 67% (30) to 82% (1, 10) and over different periods of time 3 to 12 months (1, 10, 12) 3 to 18 months (2, 13, 20), or 3 to 36 months (30). In the second year a slow and steady increase of weight as percentage of standard takes place until 4 years. Weight gain is actually above that achieved by the standard group. A similar recovery has been reported in several countries but initiated at different ages and to varying degrees (1, 2, 10, 12, 13, 19, 26, 29). The weight gains achieved by infants in the early months of life in several developing countries have been noted to be greater than by those in Europe and America (4). This was also observed in the present study but the other three measurements behaved differently. This is further evidence that changes in weight do not reflect changes in different parts of the body. The mid arm circumference, in general, follows the weight rather closely. Height and head circumference show less deviation from the standards than do the other two measurements but having fallen they remain at the level of about 95% of standard until at least the 48th month.

The results in Figs 1 and 2 and Table 5 show a high correlation between weight and mid arm circumference and between height and head circumference. Thus the members of each of these pairs of measurements have similar growth rates. It can be seen that skeletal measurements reflect better the long term effect of poor growth than do weight and mid arm circumference and that any declines in height and head circumference are irreversible changes. Consequently such ratios as weight/height are not ideal measures because recovery in weight exceeds that in height and retardation of growth may be masked in this way.

In this study females tended to be lighter than males in comparison with the standards for their own sex (Table 1). The difference

between means of height and weight of the two sexes was highly significant ($P < 0.001$). Evidence that cultural influences may favour boys has been reported in some parts of the world (9, 25, 33).

The heights, but not the other measurements, differed according to location (Table 2). The three communities are of the same ethnic stock and all are of a low socio-economic background. Further investigation of this point is indicated.

The various differences in the measurements according to season of measurement and season of birth are rather complex and do not always have a ready explanation (Tables 3 and 4). The lower weight in summer than in other seasons may be attributable to the peak of diarrhoeal disease at this time and possibly also to the effect of high temperature and humidity on food intake. There is similar evidence from many other countries (7, 15). Infants born in winter might be expected to be particularly exposed to the hazards of weaning during the summer months (7).

SUMMARY

During a period of 12 months 1 231 Lebanese Arab children of low socio-economic class, 3-48 months of age living in three locations were measured. The measurements were weight, height, head circumference and mid arm circumference. The results were expressed as percentage of international standards for boys and girls separately. The patterns of deviation from the standard values varied according to the measurement. In general for both sexes there was a falling off between 6 and 18 months greatest for weight and mid arm circumference with some recovery later and less for height and head circumference without recovery later. The possible influences of location and season of observation and birth were studied. Using these four somatic measurements an Index of Thriving was developed for the subsequent study of contrasting groups of control and failure to thrive children. The use of this

and other simple means of assessing failure to thrive in the field are discussed.

ACKNOWLEDGEMENTS

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POSTNATAL CHANGES IN SOME RED CELL PARAMETERS

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The red cell count is a basic study parameter of postnatal erythropoiesis. Yet available data are derived mostly from work done before the advent of electronic counters. Because of the error inherent in red cell counts done in the counting chamber the results were subject to considerable variation. Indeed earlier workers have come to rely mainly on the hemoglobin mass in estimating the magnitude and timing of postnatal changes in the erythron. However changes in the number of circulating red cells reflect the interplay between red cell production and destruction more accurately than do changes in the hemoglobin mass.

In the present communication some current concepts of the dynamics of erythropoiesis after birth have been reevaluated in the light of more precise data on the red cell count and a study of some other red cell parameters.

The data on the red cell count represent useful reference material for normal values in the age group 1 day to 12 weeks and are therefore reported in detail along with data on the hematocrit, hemoglobin concentration and reticulocyte counts which have been obtained concurrently. Other red cell parameters studied included red cell size and cell density distributions. The latter is a good reflection of red cell age distribution (3).

MATERIAL AND METHODS

The red cell count, hemoglobin concentration, hematocrit, reticulocyte count and red cell size distribu-

tion were followed from birth to 12 weeks in an apparently healthy full term infant population delivered in this center. Observations were made daily during the first week of life and at weekly intervals thereafter. Between 10 and 20 observations were made for each day or week. It is the practice in this hospital to clamp the cord early usually within one minute after the infant is delivered.

All counts (including the first day) were done on heel prick blood. The skin was pricked with a Medipoint blood lancet 3 mm deep and free flowing blood was aspirated. Red cell counts and cell size distribution curves were determined in an electronic counter. Hemoglobin was determined as oxyhemoglobin in a photoelectric colorimeter calibrated with a cyanmethemoglobin standard. The hematocrit was determined as microhematocrit.

The total number of circulating red cells was calculated from the mean red cell count, the mean weight for each group and a factor representing blood volume/kg body weight as determined by other workers (10, 13, 17). The figures used were 89, 87, 85, 80 and 75 ml/kg for birth, 1 week, 2-4 weeks, 5 weeks and 6-12 weeks respectively.

Red cell density distribution was determined according to Danon & Markovitz (3). Blood was drawn into a series of 20 microhematocrit capillary tubes containing mixtures of phthalate esters with specific gravity increasing in steps of 0.04 from 1.036 to 1.138. Following centrifugation a separation of the cells heavier than the phthalate mixture in each tube was achieved.

Precipitation curves were determined on the heavy and light fractions each representing 2-6% of the red cell population contained in the 2-5 extreme fractions. Macrocytes having a volume of 186 μ and above have been excluded from calculations since this fraction was found to contain also large numbers of white cells and fat particles. The mean corpuscular volume in various fractions (whole blood, heavy and light fraction) was calculated from the weighted means in each cell volume step.

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POSTNATAL CHANGES IN SOME RED CELL PARAMETERS

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The red cell count is a basic study parameter of postnatal erythropoiesis. Yet available data are derived mostly from work done before the advent of electronic counters. Because of the error inherent in red cell counts done in the counting chamber the results were subject to considerable variation. Indeed earlier workers have come to rely mainly on the hemoglobin mass in estimating the magnitude and timing of postnatal changes in the erythron. However changes in the number of circulating red cells reflect the interplay between red cell production and destruction more accurately than do changes in the hemoglobin mass.

In the present communication some current concepts of the dynamics of erythropoiesis after birth have been reevaluated in the light of more precise data on the red cell count and a study of some other red cell parameters.

The data on the red cell count represent useful reference material for normal values in the age group 1 day to 12 weeks and are therefore reported in detail along with data on the hematocrit, hemoglobin concentration and reticulocyte counts which have been obtained concurrently. Other red cell parameters studied included red cell size and cell density distributions. The latter is a good reflection of red cell age distribution (3).

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The red cell count, hemoglobin concentration, hematocrit, reticulocyte count and red cell size distribu-

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The total number of circulating red cells was calculated from the mean red cell count, the mean weight for each group and a factor representing blood volume/kg body weight as determined by other workers (10, 13, 17). The figures used were 89, 87, 85, 80 and 75 ml/kg for birth, 1 week, 4 weeks, 5 weeks and 6-12 weeks respectively.

Red cell density distribution was determined according to Danon & Markovsky (3). Blood was drawn into a series of 20 microhematocrit capillary tubes containing mixtures of phthalate esters with specific gravity increasing in steps of 0.04 from 1.036 to 1.138. Following centrifugation a separation of the cells heavier than the phthalate mixture in each tube was achieved.

Price Jones curves were determined on the heavy and light fractions each representing 2-6% of the red cell population contained in the 2-5 extreme fractions. Macrocytes having a volume of 186 μ^3 and above have been excluded from calculations since this fraction was found to contain also large numbers of white cells and fat particles. The mean corpuscular volume in various fractions (whole blood, heavy and light fraction) was calculated from the weighted means in each cell volume step.

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Table 1 Red cell values in the first 12 weeks of life

Age	No of cases	Hb g/100 ml ±SD	RBC × 10 ⁶ ±SD	HCT ±SD	MCV μ ³ ±SD	MCHC ±SD	RETIC ±SD
Days							
1	19	19.3 ± 2.2	5.14 ± 0.7	61 ± 7.4	119 ± 9.4	31.6 ± 1.9	3.2 ± 1.4
2	19	19.0 ± 1.9	5.15 ± 0.8	60 ± 6.4	115 ± 7.0	31.6 ± 1.4	3.2 ± 1.3
3	19	18.8 ± 2.0	5.11 ± 0.7	62 ± 9.3	116 ± 5.3	31.1 ± 2.8	2.8 ± 1.7
4	10	18.6 ± 2.1	5.00 ± 0.6	57 ± 8.1	114 ± 7.5	32.6 ± 1.5	1.8 ± 1.1
5	12	17.6 ± 1.1	4.97 ± 0.4	57 ± 7.3	114 ± 8.9	30.9 ± 2.2	1.2 ± 0.2
6	15	17.4 ± 2.2	5.00 ± 0.7	54 ± 7.2	113 ± 10.0	32.2 ± 1.6	0.6 ± 0.2
7	12	17.9 ± 2.5	4.86 ± 0.6	56 ± 9.4	118 ± 11.2	32.0 ± 1.6	0.5 ± 0.4
Weeks							
1-2	32	17.3 ± 2.3	4.80 ± 0.8	54 ± 8.3	112 ± 19.0	32.1 ± 2.9	0.5 ± 0.3
2-3	11	15.6 ± 2.6	4.20 ± 0.6	46 ± 7.3	111 ± 8.2	33.9 ± 1.9	0.8 ± 0.6
3-4	17	14.2 ± 2.1	4.00 ± 0.6	43 ± 5.7	105 ± 7.5	33.5 ± 1.6	0.6 ± 0.3
4-5	15	12.7 ± 1.6	3.60 ± 0.4	36 ± 4.8	101 ± 8.1	34.9 ± 1.6	0.9 ± 0.8
5-6	10	11.9 ± 1.5	3.55 ± 0.2	36 ± 6.2	102 ± 10.2	34.1 ± 2.9	1.0 ± 0.7
6-7	10	12.0 ± 1.5	3.40 ± 0.4	36 ± 4.8	105 ± 12.0	33.8 ± 2.3	1.2 ± 0.7
7-8	17	11.1 ± 1.1	3.40 ± 0.4	33 ± 3.7	100 ± 13.0	33.7 ± 2.6	1.5 ± 0.7
8-9	13	10.7 ± 0.9	3.40 ± 0.5	31 ± 2.5	93 ± 12.0	34.1 ± 2.2	1.8 ± 1.0
9-10	12	11.2 ± 0.9	3.60 ± 0.3	32 ± 2.7	91 ± 9.3	34.3 ± 2.9	1.2 ± 0.6
10-11	11	11.4 ± 0.9	3.70 ± 0.4	34 ± 2.1	91 ± 7.7	33.2 ± 2.4	1.2 ± 0.7
11-12	13	11.3 ± 0.9	3.70 ± 0.3	33 ± 3.3	88 ± 7.9	34.8 ± 2.2	0.7 ± 0.3

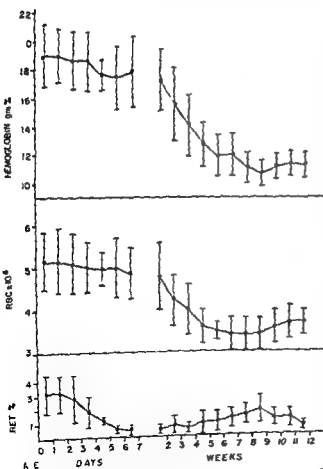


Fig. 1 Hemoglobin concentration, red cell count and reticulocyte count in the first 12 postnatal weeks. Vertical bars represent standard deviation.

RESULTS

Changes in the red cell count, hemoglobin concentration, hematocrit, MCV, MCHC and the reticulocyte count at daily intervals from day 1 to 7 and at weekly intervals from the second to the twelfth week are summarized in Table 1 and Fig. 1.

The mean red cell count on the first day of life was 5.14×10^6 . It dropped steadily, reaching a low point of 3.4×10^6 in the 7th week, remained unchanged for the following 3 weeks and showed a tendency to rise from the tenth week to the end of the period of observation.

In Fig. 2 results are expressed as the total number of circulating red cells derived from the red cell count and calculated blood volume. It can be seen that the 7th week represents the turning point from a negative to a positive balance between red cell production and destruction.

The hemoglobin concentration on day 1 was 19.3 g/100 ml. It decreased steadily, reaching a low point of 10.7 g/100 ml during the 9th week and subsequently rising to 11.3 g/100

ml during the remaining 3 weeks of observation

The MCHC on day 1 31.6% was somewhat low by adult standards (Table 1) Following a gradual increase during the first 5-6 weeks it fluctuated around 34% for the remaining period of observation The difference between the MCHC value for day 1 or day 7 and any of the values for weeks 5 to 10 was significant ($p < 0.01$) while there was no significant week-to-week variation during the period 5-12 weeks

The mean reticulocyte count on day 1 was 3.2% decreasing gradually from day 3 and reaching the 1% level on day 6 From the second to the 5th week it fluctuated between 0.5 and 1.0% A gradual rise was noted beginning in the 5th week and reaching a high of 1.8% in the 9th week. During the remaining 3 weeks of observation the reticulocyte count gradually returned to around 1% (Table 1 Fig 1)

The cell size distribution (Price Jones) curve on day 1 and day 28 is presented in Fig 3 The curves represent mean values for cell sizing obtained on 11 and 15 individual infants respectively A mean cell size distribu-

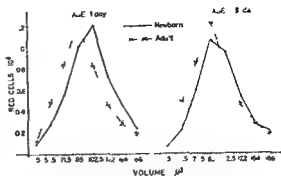


Fig 3 Mean cell size distribution (Price Jones) curves at age 1 day and 28 days Cell size distribution in adults is shown for comparison

tion curve obtained in adults is presented for comparison Both adult and newborn distribution curves are skewed to the right a phenomenon commonly observed with electronic cell sizing (2, 16) It can be seen that the distribution curve on day 1 is markedly shifted to the right As compared with the adult in whom $85\ \mu^3$ represents the peak frequency in the 1 day-old infant this frequency is $102.5\ \mu^3$ There is a considerable increase in the frequency distribution of macrocytes with a corresponding decrease in microcytes By 4 weeks the red cell size distribution curve approaches that of the adult

To follow the red cell size distribution curve as a function of age (Fig 4) the red cell population was somewhat arbitrarily divided into 3 groups

Cells having a volume of 71.5 – $102.5\ \mu^3$ were grouped together as normocytes while smaller (34.0 – $57.5\ \mu^3$) and larger (122.0 – $164.0\ \mu^3$) cells comprised the microcytic and macrocytic

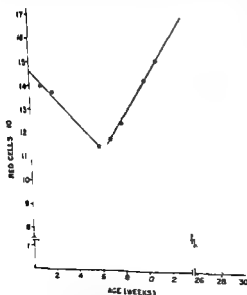


Fig 2 Total number of circulating red cells in the first 12 postnatal weeks

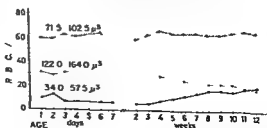


Fig 4 Changes in the percentage distribution of macrocytes, normocytes and microcytes in the first 12 postnatal weeks

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Age	No of cases	Hb gm/100 ml ±S D	RBC $\times 10^6$ ±S D	HCT ±S D	MCV μ^* ±S D	MCHC ±S D	RETIC~ ±S D
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3-4	17	14.2±2.1	4.00±0.6	43±5.7	105±7.5	33.5±1.6	0.6±0.3
4-5	15	12.7±1.6	3.60±0.4	36±4.8	101±8.1	34.9±1.6	0.9±0.8
5-6	10	11.9±1.5	3.55±0.2	36±6.2	102±10.2	34.1±2.9	1.0±0.7
6-7	10	12.0±1.5	3.40±0.4	36±4.8	105±12.0	33.8±2.3	1.2±0.7
7-8	17	11.1±1.1	3.40±0.4	33±3.7	100±13.0	33.7±2.6	1.5±0.7
8-9	13	10.7±0.9	3.40±0.5	31±2.5	93±12.0	34.1±2.2	1.8±1.0
9-10	12	11.2±0.9	3.60±0.3	32±2.7	91±9.3	34.3±2.9	1.2±0.6
10-11	11	11.4±0.9	3.70±0.4	34±2.1	91±7.7	33.2±2.4	1.2±0.7
11-12	13	11.3±0.9	3.70±0.3	33±3.3	88±7.9	34.8±2.2	0.7±0.3

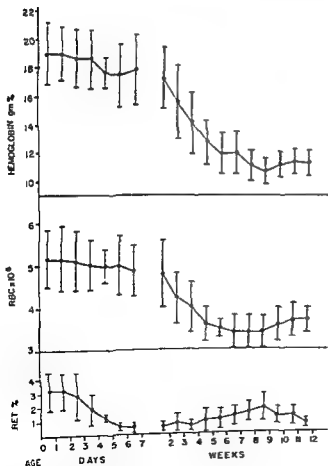


Fig 1 Hemoglobin concentration, red cell count and reticulocyte count in the first 12 postnatal weeks. Vertical bars represent standard deviation.

RESULTS

Changes in the red cell count, hemoglobin concentration, hematocrit, MCV, MCHC and the reticulocyte count at daily intervals from day 1 to 7 and at weekly intervals from the second to the twelfth week are summarized in Table 1 and Fig 1.

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The hemoglobin concentration on day 1 was 19.3 g/100 ml. It decreased steadily, reaching a low point of 10.7 g/100 ml during the 9th week and subsequently rising to 11.3 g/100

ent at birth are followed within the first few postnatal days by a markedly reduced erythropoietic activity in the marrow and a low reticulocyte count (5-14). Our results conform with this general picture. In some important details, however, our findings differ from those of earlier workers.

The time of clamping of the umbilical cord is a variable which may affect red cell values in the postnatal period (4-8). Since early clamping is practiced in this hospital, our infant population can be regarded as uniform in this respect. The source of the blood samples is also of importance, since red cell values are known to be higher in capillary than in venous blood. Therefore the red cell values obtained by us represent normal values for capillary blood in an infant population with early clamping of the umbilical cord. Indices calculated from these values such as MCHC are not affected by the source of blood. The dynamics of the red cell count or hemoglobin concentration may however vary somewhat with the source of blood especially during the first week of life.

The trimenon anemia is a widely held concept. The postnatal drop in the red cell count and hemoglobin concentration is generally believed to continue well into the third month (6-9, 19). It is however clear from our data that while this is true of the hemoglobin concentration, the low point in the red cell count is reached during the 7th week. The lag of the hemoglobin concentration behind the red cell count is accounted for by the continuing decrease in MCV.

That the 7th week represents a turning point is evident from a plot of the number of total circulating red cells (Fig. 2). Although blood volumes were not actually determined by us, calculations based on the red cell count, weight and values for blood volume obtained by other workers for the age groups in question represent a close approximation.

The data could be best fitted into two separate linear functions, the first of which covered the period from birth to the seventh

week. Assuming complete cessation of red cell production after birth, this plot would represent a red cell survival curve. The absurdity of such an assumption is evident from an extrapolation of this plot which intercepts the abscissa at about 220 days (Fig. 2). This figure is twice the normal life span of adult red cells and probably 3 times that of neonatal cells (7-11). It is therefore evident that red cells are produced in considerable numbers during the postnatal period. For the first 6 weeks, however, production falls short of compensating for factors tending to lower the red cell count, such as the short life span of the neonatal red cell and the expanding blood volume. Since the rate of weight gain and the corresponding increase in blood volume are not markedly changed after 6 weeks, the positive balance between red cell destruction and production achieved at this age can be attributed to an increase in the rate of production. An increase in the mean red cell life span may also be a contributing factor.

The reticulocyte count can be cited as further evidence for the persistence of considerable erythropoietic activity following the immediate postnatal slowdown. It should be noted that after the initial drop at the end of the first week, the reticulocyte count remained in the 0.5-1.0% range. This corresponds to the normal adult level of reticulocytosis and is far from being indicative of a depressed erythropoiesis. The rise in the reticulocyte count, which is noticeable from the 5th week on, indicates an increase in erythropoietic activity. The rise is slow, reaching its peak value at 8 weeks. Thus it is in keeping with a gradual increase in the level of red cell production rather than an abrupt "turning on" of erythropoiesis in the third month.

It has been shown that the red cell density distribution reflects cell age distribution (3, 12). As in the adult, the red cell density distribution curve in the newborn is sigmoid in shape. However, a comparison of the neonatal curve to that of adults should be approached with caution. Since neonatal red

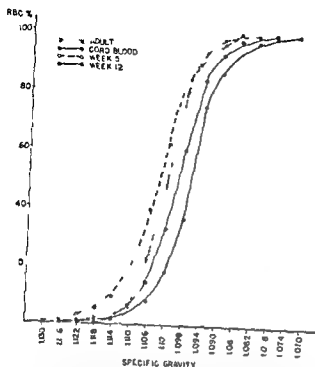


Fig 5 Mean red cell density distribution curves at birth 5 weeks and 12 weeks. A density distribution curve of adult red cells is presented for comparison.

groups respectively. It can be seen that the percentage of macrocytes remained constant over the first 6 days. On day 7 and during the second week there was actually some increase in the percentage distribution of macrocytes, followed by a steady decrease to the end of the period of observation. The microcyte population was small initially. From the 3rd week on it showed a steady increase, reciprocal to the decrease in macrocytes. The normocyte population remained constant throughout most of the period of observation.

Cumulative density distribution curves obtained at birth, 5 weeks and 12 weeks are presented in Fig 5. Typical sigmoid curves similar to those observed in adults are obtainable in the newborn. However, minor fractions of cells with specific gravity outside the normal adult span are found in the newborn. A fraction lighter than 0.090 is present in cord blood while a cell population heavier than 1.114 is found at 5 weeks.

The density distribution curve at birth lies well to the right (light side) of the adult curve. From birth to 5 weeks the curve shifts gradu-

ally to the left (heavy side), while from 5 to 12 weeks the trend is reversed and a shift back to the right is observed. At 12 weeks the density distribution curve is again situated to the right of the adult curve.

To determine the relationship between cell size and cell density distribution, 13 full term infants aged 1-28 days, 10 premature infants of 30-34 weeks gestation and 4 adults were studied. Cell sizing was done on the light and heavy fractions and revealed a preponderance of macrocytes (cells having a volume of 102.5-186.0 μ^3) in the light fraction of neonatal red cells. This tendency was more marked in red cell populations obtained from premature infants, while in adults the cell size distribution in whole blood, light or heavy fraction did not differ significantly (Tables 2 and 3).

DISCUSSION

The state of erythropoiesis at birth and the changes it undergoes during the postnatal period are well known in their general outline. At birth there is polycythemia and macrocytosis. During the postnatal period there is a gradual decrease in the red cell count, hemoglobin concentration and MCV (6). Marrow erythroid hyperplasia and reticulocytosis, pres-

Table 2 Distribution of macrocytes in various fractions of neonatal red cell

Macrocytes \pm S.D.	Fraction		
	Whole blood	Light	Heavy
Full term	75.7 \pm 7.97	86.7 \pm 11.8	63.2 \pm 13.5
Premature	71.4 \pm 8.05	83.2 \pm 13.05	58.2 \pm 8.84
Adult	37.2	37.4	37.0

Table 3 MCV in various fractions of neonatal red cells

MCV μ^3	Whole blood	Light	Heavy
Full term	112.2	129.5	103.9
Premature	120.7	146.3	111.4

percentage distribution decreased after the second week with a corresponding increase in microcytes.

The red cell density distribution curve in the neonatal period is sigmoid in shape. The range of densities is however wider than in the adult. The curve shifts gradually to the heavy side from birth to 5 weeks while from 5 to 12 weeks a shift back to the light side is observed. Macrocytes preponderate in the light fractions.

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cells differ from adult cells in their life span, the age specific gravity relationship characteristic of adult cells does not apply to the newborn. Therefore, a given specific gravity does not necessarily represent the same red cell age in the adult and the newborn. This point is emphasized by the finding of small populations of neonatal cells having specific gravities outside the normal adult span at both extreme ends of the density distribution curve (Fig. 5).

These objections do not apply when density distributions are compared within the newborn period. The shift to the heavy side from birth to 5 weeks demonstrates clearly a deficit in young cells and a preponderance of old cells during this interval. This is compatible with a situation in which cell death exceeds the production of new cells. The density distribution at 12 weeks, on the other hand, is characteristic of the rapidly growing infant, continuously expanding its blood volume. Red cell production exceeds destruction and younger cells predominate.

The nature and fate of the macrocyte population present at birth is of interest. The macrocytes may represent a primitive fetal type of red cell, differing in size as well as in other properties from cells produced later during an adult phase of erythropoiesis. The very large volume of red cells in the early stages of gestation and its diminution as gestation progresses (15) support this hypothesis. We have found (unpublished data) the population of macrocytes, especially of the very large cells, to be considerably larger in premature infants of 28–32 weeks gestation than in term infants.

Macrocytosis at birth, on the other hand, may reflect the presence of a large population of young red cells, resulting from the intensive erythropoiesis characteristic of the prenatal period. The young red cell is known to be larger and to decrease in volume with increasing age (18). Furthermore, Brecher & Stohlman (2) found that, following severe erythroid stimulation, red cells are produced

which are macrocytic and that these stress macrocytes have a shortened life span. Our finding of a predominance of macrocytes in the lighter, i.e. younger, fractions is consistent with this latter concept. It should be noted however, that early fetal red cells may represent a qualitatively different population and have densities lower than adult cells of corresponding age.

Some increase in the percentage of macrocytes was observed during the first week of life. A similar increase was found by Wunderlich & Hinkel (19) and may be explained by the maturation and release into the peripheral blood of erythroid elements present in the marrow at birth. From the second week on the number of macrocytes decreases gradually and a simultaneous increase in microcytes takes place. Whether this change in the cell size distribution reflects elimination of short lived stress macrocytes and their replacement by a cell population of smaller size or a decrease in size associated with cell aging cannot be answered with certainty from our data. The gradual postnatal increase in MCHC is consistent with cell shrinking due to aging of the predominantly macrocytic cell population present at birth and would therefore favor the latter alternative.

SUMMARY

The red blood picture was studied in detail from birth to 12 weeks. The low point in the red cell count was reached in the seventh week which represented the turning point from a negative to a positive balance between cell production and destruction. From the dynamics of the total number of circulating red cells it was concluded that although erythropoiesis slows down after birth a considerable amount of red cell production takes place during the first few postnatal weeks.

The MCHC increased significantly over the first 5–6 postnatal weeks and remained constant thereafter.

Macrocytes predominated at birth. Their

percentage distribution decreased after the second week with a corresponding increase in microcytes

The red cell density distribution curve in the neonatal period is sigmoid in shape. The range of densities is however wider than in the adult. The curve shifts gradually to the heavy side from birth to 5 weeks, while from 5 to 12 weeks a shift back to the light side is observed. Macrocytes preponderate in the light fractions.

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CYSTATHIONINURIA IN A WELL BABY POPULATION

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Cystathionine is a sulphur-containing amino acid, classed as non-essential since it is formed from methionine. It normally occurs in tissue proteins, is found in trace amounts in blood and urine, and is not significantly resorbed by the renal tubule. A defect in the enzyme cystathioninase which cleaves cystathionine has been correlated with the appearance of large amounts of cystathionine in the blood and in the urine (10). The disorder is apparently inherited as a Mendelian recessive character. The reports of 14 patients suffering from this disorder have been summarized (14). Cystathionine has also been found in smaller amounts in the urine of some of the relatives of these individuals. These cystathionine excretors are presumptively heterozygous for the enzymic defect.

Cystathioninuria has been described in association with pyridoxine deficiency states (15) with gross liver failure (11) and with neuroblastoma (9).

More recently cystathioninuria, homocystinuria and hypomethioninaemia have been demonstrated in an infant with a defect in the activity of the homocysteine remethylating enzyme (12). This is probably due to an abnormality in vitamin B₁₂ metabolism.

Von Studnitz (20) found cystathioninuria in 6 out of 230 infants hospitalised for investiga-

tion of suspected metabolic disorders, convulsions or mental retardation.

There has been to date no survey of a significant number of normal infants to determine the incidence of excess urinary cystathionine in an otherwise unselected population.

MATERIAL AND METHODS

In the course of the urinary metabolic screening survey carried out in New South Wales (19) the urine of 35 809 infants was examined over a 6 month period by means of one way paper chromatography and ninhydrin staining. Suspected abnormal cystathionine excretion was further investigated by high voltage electrophoresis thin layer chromatography in two dimensions and by its reaction with iodoplatinate (16). Quantitative studies were made on a Beckman Unicrom amino acid analyser. Fasting plasma amino acids were quantitated after removal of the proteins with picric acid (8).

RESULTS

Cystathioninuria was recognised in 15 infants of the 35 809 screened at approximately 6 weeks of age. Analysis of follow up urine specimens obtained when the fifteen infants were approximately 5 months old revealed that eleven infants (5 males and 6 females) showed no demonstrable cystathioninuria, 2 male infants showed traces of cystathionine in the urine while a further 2 males (D M and R S) showed a persistent and heavy cystathioninuria.

Enquiries of the parents of these 15 children and of their physicians failed to elicit a history

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Table 1 *Amino acid analysis of liquid urine samples in case 1*

Age	Treatment	Cystathionine (mg) per g creatinine	Homocystine (mg) per g creatinine
70 weeks	None	1943	150
22 weeks	100 mg pyridoxine daily for 2 weeks	55	Nil
9 months	Pyridoxine suspended for 4 weeks prior to test	1043	Nil
10 months	Methionine 100 mg per kg body weight (single dose)	1309	12
17 months	500 mg pyridoxine daily for 4 weeks	21	Nil

of abnormality in these infants. Three infants were examined by one of us (PGP) and no clinical abnormalities were revealed. Further studies on the 2 infants with persistent cystathioninuria were undertaken.

CASE REPORTS

Case 1 (D M S315085 male) was born of unrelated parents and weighed 3400 g at full term. No neonatal problems were encountered and he was artificially fed from birth. At 7 weeks of age a urine sample revealed excessive cystathionine excretion which was confirmed by a repeat test at 16 weeks. The child was alert and responsive but its motor milestones were slightly delayed, namely sitting alone at 8 months and beginning to crawl at 12 months.

A positive cyanide nitroprusside test (4) was obtained on a liquid urine sample obtained at 20 weeks. Quantitative amino acid analysis revealed elevated levels of both cystathionine and homocystine in the urine while cystine excretion was normal (Table 1). Cystathionine (0.050 μ moles/ml) was detected in fasting plasma at 20 weeks.

Pyridoxine hydrochloride (250 mg daily) was given orally and a urine test 2 weeks later

showed a marked reduction in cystathionine and no demonstrable homocystine in the urine. At 8 months of age pyridoxine was suspended for 4 weeks and the concentration of cystathionine in the urine again rose (Table 1). Homocystine was not demonstrated in the urine. Haematological studies revealed no abnormality. The vanillylmandelic acid level in the urine was normal and no methylmalonic acid was detected.

Cystathionine was not detected in early morning urine specimens of the parents or of the 3 siblings.

After 500 mg of pyridoxine daily for 4 weeks the cystathionine excretion at the age of 12 months was reduced to almost normal levels.

Loading tests. When daily administration of pyridoxine to the propositus had been suspended for 4 weeks he and his parents were given methionine (100 mg/kg body weight) with the evening meal and early morning urine specimens were collected the next day. Cystathionine was detected in trace quantity in the urine samples from the parents. In the infant, then 10 months of age, cystathionine excretion rose to over 1 g/g creatinine and homocystine was again demonstrable. The cyanide nitroprusside test was weakly positive.

Case 2 (R S S315875 male) the firstborn of healthy and unrelated parents weighed 3020 g at full term. The baby was artificially fed, thrived normally and achieved his developmental milestones at normal times. Excessive cystathionine excretion was first demonstrated at 5 weeks of age and was confirmed at 19 weeks. The amino acids were quantitated in a liquid urine sample at 21 weeks (Table 2) and cystathioninuria was confirmed. Glycine excretion was elevated but homocystine was not demonstrated.

Pyridoxine hydrochloride (250 mg daily) administered orally was given by mouth for 2 weeks and the cystathionine excretion was reduced but the excretion of glycine increased. This elevation of glycine excretion was accompanied by excesses in proline and hydroxypro-

line excretions. Four weeks after the cessation of pyridoxine treatment the cystathionine excretion returned to pretreatment levels whereas glycine excretion returned to normal values.

Fasting plasma amino acids quantitated at 21 weeks revealed an elevated cystathionine level ($0.027 \mu\text{moles/ml}$), normal glycine values, and absence of homocystine was demonstrated.

Urine samples from the parents showed an excretion of cystathionine in the father of 60 mg/g of creatinine on one occasion, but no demonstrable cystathioninuria in the mother.

Loading tests (as in case 1) revealed a trace of cystathionine in the mother's urine but none was demonstrated in the father's urine on this occasion.

DISCUSSION

The incidence of cystathioninuria in a population of healthy infants has not been determined previously. The detection of excessive cystathionine excretion by the present method clearly underestimates its incidence. The lack of specificity obtained by ninhydrin staining of the chromatograms makes a considerable elevation of cystathionine necessary before an abnormality is suspected. It would appear likely, however, that infants with considerable cystathioninuria would be detected.

Genetic studies (7, 21), which have been conducted on the families of patients who are excreting large amounts of cystathionine and whose plasma showed elevated levels of cystathionine, suggest that these individuals are homozygous for an enzyme defect. The heterozygotes may show a slight excess of urinary cystathionine and this may become more apparent after a loading dose of methionine, the precursor of cystathionine (13).

The defect in the enzyme cystathionase in the liver of the presumptive homozygous individuals has been shown to respond *in vitro* to an excess of pyridoxine (6).

The two infants ascertained who showed both a heavy cystathioninuria and cystathioninemia responding to pyridoxine administration,

Table 2 Amino acid analysis of liquid urine samples in case 2

Age	Treatment	Cystathionine (mg) per g creatinine	Glycine (mg) per g creatinine
21 weeks	None	1909	358
23 weeks	100 mg Pyridoxine daily for 2 weeks	377	585
27 weeks	Pyridoxine suspended for 4 weeks prior to test	836	84
Normal		125	100

would appear to be homozygous for this enzyme defect. The demonstration of cystathioninuria in each of the four parents, at one time or other, indicates that they may be heterozygous for this enzyme defect.

The clinical significance of homozygous cystathioninuria has been rendered obscure by the methods employed previously in ascertainment of cases. Of the described cases only 2 patients were subjected to fortuitous urinary amino acid chromatography (13). The combination of these patients, the normal relatives of ascertained patients (5, 14), and the present patients suggests that if this condition has some clinical significance then it has to date, escaped recognition. The suggestion that cystathioninuria is a benign disorder (6, 13) is supported by the present study.

The study of Scriver & Hutchison (15), confirmed in man that vitamin B₆ deficiency will produce cystathioninuria. No evidence of such deficiency was obtained in any of the infants studied in the present series. Cystathioninuria has also been reported in patients with galactosaemia, hepatoblastoma and neuroblastoma. There has been no evidence to suggest these possibilities in any of the infants in the present series.

The infants in whom transient cystathioninuria has been demonstrated may be heterozygotes for this condition or they may have had a transient vitamin B₆ deficiency. These possi-

bilities remain unexplored. Similarly the two infants in whom trace amounts of cystathionine persisted in the urine may be heterozygotes. It is less likely however, that they are vitamin B₆ deficient as they were receiving adequate diets in a good state of nutrition, and were not receiving any recognised pyridoxine antagonists.

The presence of a positive cyanide nitroprusside test in a patient with cystathioninuria has previously been reported (3, 12, 18). One case was characterised biochemically by the presence of hypomethioninaemia and the excretion of methylmalonic acid in the urine (12). This disorder has been shown to be a different entity from those presently under consideration where neither of these conditions were present. In Berlow's patient no cause for the positive cyanide nitroprusside test could be found and in particular homocystine was not present in the urine (2). Both the cystathioninuria and the positive cyanide nitroprusside test responded favourably to the administration of pyridoxine in Berlow's case. Tada *et al* (18) found a small amount of homocystine insufficient to cause a positive cyanide nitroprusside reaction in the urine of his cystathioninuric patient. In this case the biochemical abnormalities did not respond to pyridoxine.

The amount of homocystine in the urine of our case 1 (D M) was much higher than in Tada's patient and was of sufficient quantity to produce a positive cyanide nitroprusside test. This finding was associated with very high levels of both plasma (0.050 μmol per ml) and urinary cystathionine (1.943 mg per g of creatinine). After a course of pyridoxine therapy the cystathionine in the urine fell markedly and the homocystine disappeared. Although cystathionine excretion again rose after pyridoxine was suspended homocystine did not reappear in the urine until a methionine load test further increased the cystathionine excretion.

Although it has been shown in experimental animals that cystathioninuria and homocystinuria can be caused by vitamin B₆ deficiency

(1) the diet of D M has always been adequate. It has been estimated that at the time when homocystinuria was present he was receiving 0.5 mg of vitamin B₆ daily from his milk formula alone in addition to that received from other dietary sources such as cereals. Also no other clinical effects of vitamin B₆ deficiency such as convulsions or anaemia have been observed. The finding of a normal urine taurine level of 22 mg per g creatinine argues against vitamin B₆ deficiency being the cause of this child's biochemical abnormalities (17).

SUMMARY

Screening of 35 809 infants by one way chromatography revealed 15 cases of excessive cystathionine excretion. Evidence is presented to suggest that two of these are homozygous for primary cystathioninaemia. Homocystinuria was present transiently in one of these two infants. It is concluded that cystathioninuria is a benign disorder and appears to be much more common than previously recognised.

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THE THIRD FONTANELLE

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In a continuing study of Down's syndrome in the newborn infant the presence of the third fontanelle was noticed fairly frequently. This high incidence was also noticed in infants presenting with the congenital rubella syndrome. It was therefore decided to make a study of this particular single defect in the newborn infant in the variety of conditions that occur in this hospital. It has been shown that major abnormalities are more frequent in infants presenting with minor congenital defects (6).

The third fontanelle is a bony defect along the sagittal suture about 2 cm anterior to the posterior fontanelle (3, 4, 5). It is not a true fontanelle being related exclusively to the parietal bones. This fontanelle can often be seen on X-rays as a depression in the contour on a lateral view (Fig. 1) and a diamond shaped defect on the antero-posterior view which however is often obscured by overlying bones. It can be seen at autopsy in some infants.

MATERIAL AND METHOD

In the Kandang Kerbau Hospital Singapore where this study was made over 31 000 infants were delivered in 1969. This study was made over a period of 6 months. Infants in the first week of life were selected at random and examined clinically to detect any physical defect.

The infants were then divided into the normal group with no physical defects which served as controls and a second group consisting of infants in whom some abnormality was detected. A separate group of infants with Down's syndrome was also included in this study; this group was collected over a longer period of time as compared with the controls. During this period too a fairly large number

of infants were noticed to be suffering from the expanded rubella syndrome; these infants were also included in this study.

The presence of the third fontanelle was assessed by palpating the skull of the infant along the sagittal suture from the anterior fontanelle to the posterior fontanelle for the presence of a defect about 1/2 to 2/2 cm anterior to the posterior fontanelle. The procedure was repeated carefully twice. Only definite defects were accepted as evidence of the presence of the third fontanelle. Notches and doubtful findings were excluded in this study. The defect was classified as large if the coronal diameter was more than 1 cm and small if the diameter was 1 cm or less. The results were subjected to statistical analysis for significance.

RESULTS

Altogether 1 930 controls were examined consisting of 1 518 Chinese, 280 Malays, 120 Indians and 12 of mixed or other ethnic groups. The incidence in the separate ethnic groups did not show significant differences; all the infants of different ethnic groups were therefore studied together. The incidence according to weight was highest in the group weighing 2 001 to 2 500 g (Table 1). The incidence of the third fontanelle seemed to be unusually high in the group presenting with the congenital rubella syndrome (Table 2) compared with the total overall incidence of the controls ($p < 0.001$) or the weight group of the controls with the maximum incidence ($p < 0.001$). This significantly high incidence was also present in the group with Down's syndrome ($p < 0.001$).

Of the 22 infants with the expanded rubella syndrome that were examined, 16 were less

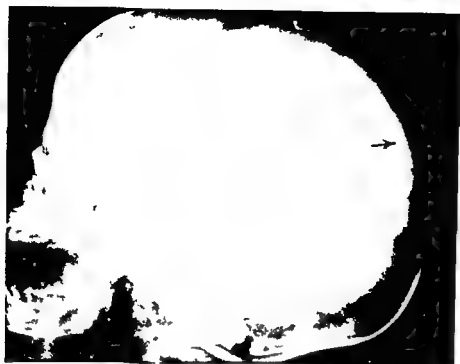


Fig 1 Third fontanelle indicated (arrow) in lateral view of the skull

than 2 000 g in weight at birth and 3 between 2 001 and 2 500 g only 3 being more than 2 500 g. Twenty of these infants were over 38 weeks' gestation at delivery.

The size of the third fontanelle was very variable: the largest in the control group was 3 cm while in the congenital rubella group the largest third fontanelle (2.8 cm) was found in a 1 290 g small for date infant. In the infants with Down's syndrome the largest fontanelle was 3.2 cm.

The incidence of the third fontanelle in the group consisting of various abnormal conditions (Table 3) was higher than that in the con-

trols but this association was not significant ($0.1 > p > 0.05$). With regard to the individual conditions in this group the numbers involved were too small to allow any definite conclusions to be drawn.

DISCUSSION

The parietal bone ossifies in membrane from one or two centres in the region of the future parietal eminence at about the seventh or eighth week of intra uterine life (7). A network of fine bony trabeculae spreads from the region of the eminence in the form of radiat-

Table 1 Incidence in normal infants

Weight group (g)	Total seen	No. with the third fontanelle					
		Actual numbers			Incidence (per 1000)		
		Large	Small	Total	Large	Small	Total
1 501 and below	40	1	1	2	25	25	50
1 501-2 000	132	9	12	21	68	91	159
2 001-2 500	303	36	27	63	120	90	210
2 501-3 000	561	47	43	90	84	77	161
3 001-3 500	599	41	27	68	69	45	114
3 501-4 000	233	13	9	22	56	39	95
4 001 and above	62	4	0	4	66	0	66
Total	1 930	151	119	270	78	62	140

Table 2 Incidence in abnormal infants

		No. with the third fontanelle			Incidence (per 1000)		
	Total seen	Actual numbers					
		Large	Small	Total	Large	Small	Total
Down's syndrome	11	35	14	49	416	168	584
Congenital rubella syndrome	22	14	5	19	636	227	863

ing bony spicules. The spread towards the central plane is slow and the defect in this region constitutes the third fontanelle. The third fontanelle normally disappears by the seventh month of fetal life (3) but it occasionally persists as the third fontanelle in infancy.

The incidence of the third fontanelle has been found to be 44-64 per 1 000 (3, 4, 5). In the present study an overall incidence of 140 per 1 000 is very much higher than the above mentioned investigations. This reason for this great disparity is not understood, however the present incidence is much lower than that arrived at by Adair & Scammon (1). These authors by tracing the sagittal suture on a piece of linen placed over it found a very high incidence of 307 per 1 000 in a study of 241 infants in the first month of life more than twice the number of the present study. The detection of the third fontanelle by the method of palpation is not difficult though cases of craniotabes may present some difficulties. With careful light, and repeated palpation of the sagittal suture in such cases a correct diagnosis can be arrived at reliably besides the incidence of craniotabes is not high enough to influence the overall results.

In another study (3) a higher incidence was found in prematures though this difference was not significant. In the present study no such incidence was found. The highest incidence was found in the group weighing from 2 001 g to 2 500 g. The group weighing 1 500 g and less in fact had the lowest incidence.

In Down's syndrome there was an unusually high incidence in the presence of the third fontanelle compared with the overall incidence

of the controls ($p < 0.001$) or compared with the weight group with the highest incidence ($p < 0.001$). This finding is in agreement with that of Chemke & Robinson (3) but differs from that of Hoyle & Franklin (4). Carter & MacCarty (2) mentioned that it is often present in Down's syndrome while Penrose & Smith (8) have commented on its occasional presence in this syndrome. The present study of 84 newborns with Down's syndrome shows quite definitely the unusual frequency of the phenomenon.

The incidence of the third fontanelle seems to be also remarkably high in the congenital rubella syndrome. This is believed to be the first occasion where this microsign has been shown to be unusually high in this condition though Chemke & Robinson (3) mentioned its presence in one infant with this condition. Its frequency is so high that it can be regarded as

Table 3 Incidence in other abnormal infants

	No with 3rd fontanelle		
	No seen	Large	Small
Exomphalos	3	1	0
Ellis van Creveld syndrome	1	1	0
Pierre Robin syndrome	2	1	1
Congenital syphilis	1	0	0
Primary macroglossia	3	0	0
Arnold Chiari syndrome	1	0	0
Partial albinism	2	0	0
Pretauricular tag	5	1	1
Skin aplasia scalp	4	1	0
Anophthalmia	1	0	0
Oesophageal atresia	1	0	0
Trisomy 17-18	1	0	0
Chordee	2	0	0
Neonatal teeth	4	1	0
Total	31		8

an almost constant feature of the syndrome. Twenty of the infants were full term though the majority were below 2 000 g in weight. Perhaps this intra uterine growth retardation also interfered with the ossification of the parietal bone resulting in the presence of the third fontanelle. No definite cases of other forms of intra uterine infection except one of congenital syphilis were available for examination during this period, however it has been shown (3) that the IgM levels were not raised in cases presenting with it. This would indicate that the third fontanelle is associated with congenital rubella rather than with intra uterine infection in general.

In the group of infants with various other abnormalities, the incidence of the third fontanelle was increased but this was not significant statistically. As for the individual conditions, within this group, further studies on larger numbers are necessary before any definite conclusions can be drawn.

SUMMARY

One thousand, nine hundred and thirty apparently normal newborn infants were examined for the presence of the third fontanelle. In addition other newborn infants with abnormalities were also screened for the same defect.

The incidence of the third fontanelle was high, in this study, among the normals, compared with past studies. The incidence seemed highest in the group weighing 2 001 to 2 500 g.

In the abnormal group, the incidence was very significantly raised in infants suffering from the congenital rubella syndrome or Downs syndrome, it was raised but not significantly in infants presenting with various other abnormalities.

The presence of the third fontanelle should alert the examiner to the possibility of other defects in the infant.

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THE HEAD CIRCUMFERENCE IN INFANTS AND OTHER MEASUREMENTS TO WHICH IT MAY BE RELATED

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The routine examination of a baby in a private house welfare clinic or hospital should include the measurement of the maximum head circumference for the head size reflects the growth of the cranial contents. If the growth of the brain is defective the head is likely to be small and if there is hydrocephalus megalencephaly or hydranencephaly the head is likely to be large. Smallness of the head may be a valuable pointer to mental subnormality and a large head to hydrocephalus and so to the appropriate treatment.

A small or large head could be merely a familial feature or a reflection of the size of the child for one would expect a large child to have a larger head than a small child and a small child to have a smaller head than a large child. Hence one must always relate the size of the head to the size of the baby. Two of us (2) showed that there is a strong relationship between the head circumference and the weight. In this paper we have set out to determine whether the head circumference is better related to the weight, chest circumference, supine length or crown rump length.

MATERIAL AND METHODS

Anthropometric data concerning 50 boys and 56 girls living in Sheffield were collected by one trained nurse for the purpose of the study. Measurements were made at birth, 6 weeks, 3 months and 6 months.

The maximum head circumference was measured by a cloth tape measure which was repeatedly checked for accuracy.

The chest circumference was measured by the tape around the chest at the level of the xiphi sternal joint. The supine length and crown rump length were measured on a supine length table. The nude weight was recorded.

Tables 1 and 2 show the mean and standard deviation of the head circumference, weight, chest circumference, supine length and crown rump length. Eight boys and two girls had a low birth weight (≤ 2500 g) and this accounts for the lower mean birth weight in boys than girls.

The correlation coefficient and the regression equation (with its standard error) were determined between head circumference and each parameter at the four different ages in each sex separately (Tables 3 and 4). The 6 week follow up included all children aged 6 weeks ± 2 weeks, the 3 months and 6 months follow ups included all children aged 3 months and 6 months ± 3 weeks.

RESULTS

From Tables 3 and 4 we observe that the correlation and the regression coefficients of the head circumference on each parameter are highest at birth (especially in boys) and at 6 weeks and are then less.

The correlation of head circumference and gestational age was low at birth in both sexes probably because of the difficulty in assessing the gestational age (for boys $r=0.416$ for girls $r=0.447$).

The head circumference showed the highest correlation with the weight in girls at all ages studied while in boys it showed the highest correlation with the weight at birth and at 3 months with the chest circumference at 6 weeks and 6 months.

Table 1 Means and standard deviations of different parameters at the different follow ups in boys

	Head circumference (cm) mean \pm S D	Weight (kg) mean \pm S D	Chest circumference (cm) mean \pm S D	Supine length (cm) mean \pm S D	Crown rump length (cm) mean \pm S D
Birth (n=50)	34.4 \pm 1.5	3.172 \pm 0.572	30.8 \pm 2.0	48.7 \pm 2.1	33.1 \pm 2.0
Six weeks (n=37)	38.1 \pm 1.7	4.622 \pm 0.677	36.3 \pm 2.8	52.9 \pm 2.1	36.1 \pm 1.9
Three months (n=43)	41.1 \pm 1.8	6.497 \pm 0.932	41.3 \pm 2.7	58.0 \pm 2.8	39.9 \pm 2.1
Six months (n=50)	44.3 \pm 1.9	8.550 \pm 1.147	45.2 \pm 2.9	64.9 \pm 2.4	43.6 \pm 1.7

Table 2 Means and standard deviations of different parameters at the different follow ups in girls

	Head circumference (cm) mean \pm S D	Weight (kg) mean \pm S D	Chest circumference (cm) mean \pm S D	Supine length (cm) mean \pm S D	Crown rump length (cm) mean \pm S D
Birth (n=56)	34.2 \pm 1.3	3.211 \pm 0.486	31.0 \pm 1.7	48.1 \pm 2.1	33.1 \pm 1.6
Six weeks (n=41)	37.8 \pm 1.2	4.372 \pm 0.560	35.8 \pm 2.0	52.4 \pm 1.6	35.8 \pm 1.6
Three months (n=52)	39.9 \pm 1.2	5.727 \pm 0.745	39.5 \pm 2.0	56.4 \pm 2.4	38.8 \pm 2.2
Six months (n=56)	42.8 \pm 1.3	7.609 \pm 0.973	43.5 \pm 2.3	62.8 \pm 2.3	42.1 \pm 1.7

Table 3 Correlation coefficient and the regression equation (with its standard error) of head circumference or other parameters in boys

r = correlation coefficient Reg. eq. = regression equation S.E. = standard error

		Head circumference and weight	Head circumference and chest circumference	Head circumference and supine length	Head circumference and crown rump length
Birth	r	0.818	0.738	0.792	0.789
	Reg. eq. S.E.	$y = 27.693 + 2.104x$ 0.847	$y = 17.282 + 0.555x$ 0.993	$y = 7.375 + 0.555x$ 0.899	$y = 15.580 + 0.568x$ 0.904
Six weeks	r	0.775	0.824	0.734	0.746
	Reg. eq. S.E.	$y = 28.997 + 1.978x$ 1.093	$y = 19.873 + 0.503x$ 0.978	$y = 6.664 + 0.595x$ 1.174	$y = 13.570 + 0.680x$ 1.151
Three months	r	0.670	0.585	0.474	0.563
	Reg. eq. S.E.	$y = 32.980 + 1.255x$ 1.297	$y = 25.200 + 0.386x$ 1.417	$y = 23.742 + 0.300x$ 1.538	$y = 22.694 + 0.462x$ 1.444
Six months	r	0.532	0.513	0.461	0.574
	Reg. eq. S.E.	$y = 36.908 + 0.867x$ 1.583	$y = 29.373 + 0.330x$ 1.604	$y = 20.735 + 0.364x$ 1.659	$y = 17.121 + 0.624x$ 1.530

Table 4 Correlation coefficient and the regression equation (with its standard error) of head circumference on other parameters in girls

r = correlation coefficient Reg. eq. = regression equation S.E. = standard error

		Head circumference and weight	Head circumference and chest circumference	Head circumference and supine length	Head circumference and crown rump length
Birth	r	0.705	0.643	0.554	0.571
	Reg. eq.	$y = -8.178 + 1.870x$	$y = 19.008 + 0.489x$	$y = 17.913 + 0.338x$	$y = 19.154 + 0.454x$
	S.E.	0.913	0.987	1.072	1.057
Six weeks	r	0.789	0.716	0.618	0.581
	Reg. eq.	$y = 30.100 + 1.790x$	$y = 21.794 + 0.446x$	$y = 13.237 + 0.468x$	$y = 21.294 + 0.460x$
	S.E.	0.767	0.866	0.975	1.009
Three months	r	0.664	0.584	0.636	0.534
	Reg. eq.	$y = 33.947 + 1.047x$	$y = 26.225 + 0.347x$	$y = 21.369 + 0.330x$	$y = 28.901 + 0.484x$
	S.E.	0.879	0.955	0.908	0.994
Six months	r	0.600	0.535	0.493	0.443
	Reg. eq.	$y = 36.455 + 0.88x$	$y = 28.956 + 0.317x$	$y = 24.440 + 0.292x$	$y = 28.404 + 0.341x$
	S.E.	1.073	1.133	1.167	1.202

In girls the head circumference correlated next best with the chest circumference at birth 6 weeks and 6 months and in boys at 3 months. The correlation of head circum-

ference and supine length or crown rump length was not so high.

Since the weight and chest circumference showed the highest correlation with the head

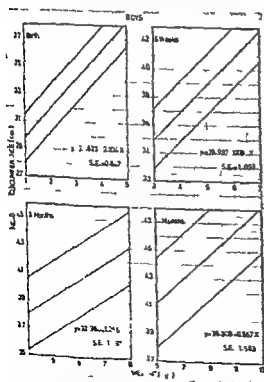


Fig. 1 Regression lines \pm standard errors of head circumference on weight in boys.

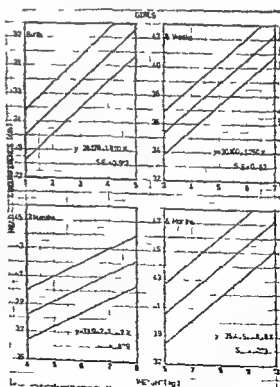


Fig. 2 Regression lines \pm standard errors of head circumference on weight in girls.

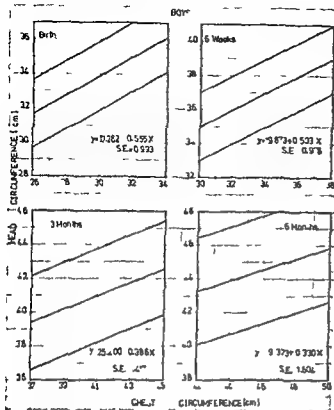


Fig 3 Regression lines ± 2 standard errors of head circumference on chest circumference in boys

circumference at most of the ages studied and because they are easily measured graphs were drawn (Figs 1, 2 3 and 4) showing the linear regression line ± 2 standard errors of head circumference on weight or chest circumference at each age and in each sex separately. If the actual head circumference is plotted on the graph appropriate for age and sex and if it lies within the limits of ± 2 standard errors (within 95% confidence limits), it is considered to be within normal limits in conjunction with other relevant clinical features. The expected head circumference can be determined directly from the graphs (Figs 1, 2 3 and 4) or by substituting weight or chest circumference for x in the regression equations (Tables 3 and 4).

DISCUSSION

The head circumference correlates highly with the body size in the first 6 months of life. It is useful to use only one graph, appropriate

for the age and sex, relating head circumference to weight or chest circumference, instead of using two graphs relating each parameter to chronological age. Illingworth & Lutz (2) stated that the relationship between head circumference and weight is uncertain in cases of failure to thrive and malnutrition, the head tending to be larger than usual in relation to the weight. Dean (1) showed that the head circumference and chest circumference are the least affected by malnutrition. In the present study, the chest circumference shows a high correlation with head circumference, nearly as high as that of the weight.

In conclusion, it would seem that for routine purposes the head circumference should be related to the baby's weight and if desired to the chest circumference.

SUMMARY

Head circumference was related to weight, chest circumference, supine length and crown-rump length at birth, 6 weeks, 3 months and 6 months in fifty boys and fifty-six girls.

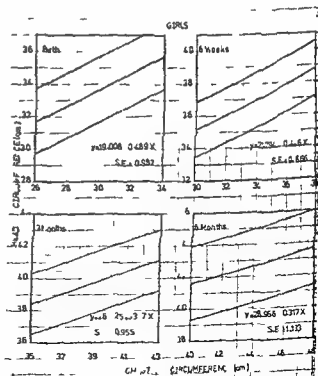


Fig 4 Regression lines ± 2 standard errors of head circumference on chest circumference in girls

1 The head circumference is highly correlated with body size especially at birth and 6 weeks

2 The head circumference shows the best correlation with weight and chest circumference particularly the weight in both sexes

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We are grateful to Professor J Knowelden and his staff of the Department of Preventive Medicine and Public Health for help with the statistical analysis. We also wish to thank Dr Ronald Gordon for permission to measure babies at the Northern General Hospital and Mrs E. Miller SRN for making the measurements

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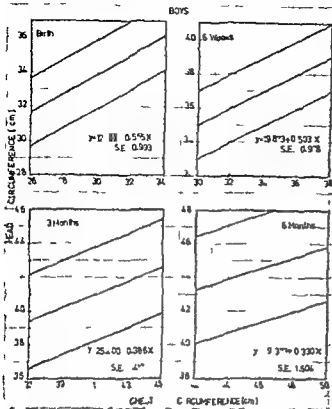


Fig. 3 Regression lines ± 2 standard errors of head circumference on chest circumference in boys

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In conclusion it would seem that for routine purposes the head circumference should be related to the baby's weight, and if desired to the chest circumference.

SUMMARY

Head circumference was related to weight, chest circumference, supine length and crown-rump length at birth, 6 weeks, 3 months and 6 months in fifty boys and fifty six girls.

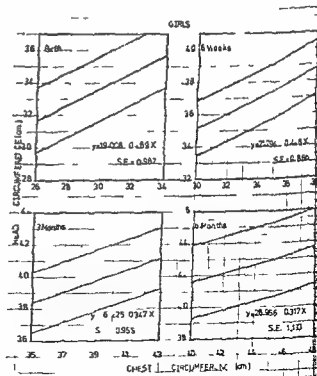


Fig. 4 Regression lines ± 2 standard errors of head circumference on chest circumference in girls



Fig 1 Duodenal juice alkaline phosphatase (DAP) activity in rachitic children (RC) and control children (CC)

from the different sources can be separated by electrophoresis (4, 6). Intestinal alkaline phosphatase is found in the serum of only one fifth of the normal adult population and when present it contributes 15 to 30% of the total activity (9). This most likely originates from the intestinal mucosa (5).

Although some decrease of this enzyme activity in the jejunum and kidney was suggested by Cheesman et al in their experimental work on vitamin D deficient rats no changes could be found in the duodenum (2). By assaying the different tissues for alkaline phosphatase activity in rachitic rats we have confirmed their findings with the exception of jejunal alteration (8). Neither was any difference demonstrated between the DAP activities of rachitic children and those of the control subjects. Duodenal juice alkaline phosphatase activity probably derives from the intestinal mucosa and hepatobiliary tree. Since the alkaline phosphatase activity of the duodenal mucosa and the liver is not altered in the vitamin D deficient rats (2, 8) our findings in children are not unexpected.

Since it is known that serum alkaline phosphatase activity may not be increased in malnourished children with rickets (10) the DAP activity of five malnourished rachitic children was compared with our control values and

no statistically significant difference could be found ($p > 0.05$). When the serum alkaline phosphatase values of our rachitic cases were compared with the corresponding DAP activities no correlation was found either. As mentioned above, we have shown that leukocyte alkaline phosphatase activity in children with rickets due to vitamin D deficiency is not significantly different from the control values (7).

From these results it might be suggested that the characteristic serum alkaline phosphatase rise in rickets is not a reflection of changes of this enzyme in all tissues where alkaline phosphatases are present. Another discrepancy between this enzyme level in serum and tissue has been reported in hypophosphatasia where serum level is markedly diminished in spite of normal activity (3, 11) in duodenal juice and mucosa. However the isoenzyme technique will probably be more valuable for a study of these problems.

SUMMARY

Duodenal juice alkaline phosphatase activity of 17 children with rickets was assayed and not found to be different from the control values. Malnutrition did not seem to affect this activity.

ACKNOWLEDGEMENT

This investigation was supported from the Scientific and Technical Research Council of Turkey. Our sincere thanks to Dr A. Kutsal of the Department of Statistics, Hacettepe University for statistical analysis.

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ALKALINE PHOSPHATASE ACTIVITY OF DUODENAL JUICE IN RICKETS DUE TO VITAMIN D DEFICIENCY

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Alkaline phosphatase is one of the first enzymes to be explored in human disease and its clinical significance is fairly well known. The rise of serum alkaline phosphatase along with the decrease of serum phosphorus level, is a characteristic finding in rickets but tissue phosphatase activity in this disease has not been investigated (4). It is surprising to find that although this enzyme activity in the tissue is fairly well documented in hypophosphatasia (1, 3, 11) which is comparatively rare and more recently described condition, there is very little in the literature about this activity in rickets.

In a previous publication we described studies on the leukocyte alkaline phosphatase level in children with rickets due to vitamin D deficiency (7). In the present study the duodenal juice alkaline phosphatase levels (DAP) were assayed in patients with rickets due to vitamin D deficiency and compared with those of normal, age matched controls.

MATERIAL AND METHODS

Duodenal juice was obtained for control values from 10 children three to 24 months of age who were admitted to the hospital for minor surgical procedures. They had no evidence of rickets on clinical, radiological or chemical examination and they were free of apparent infections. There were 17 patients with rickets 5 of whom were also malnourished. Twelve of these had acute infections of varying types. The ages of these patients ranged from 50 days to 15 months. In all cases rickets was confirmed by X-ray examination of the wrists and marked eleva-

tion of serum alkaline phosphatase was found in eight of them.

The alkaline phosphatase activity of the juice was determined by the technique of Valentine & Beck (12), using a Coleman Junior spectrophotometer (at 660 μ) for the Beckman spectrophotometer. Instead of a leukocyte suspension the same amount of duodenal juice (0.3 ml) was added to an incubation mixture which did not contain saponine. With the exception of sample and reagent blanks each determination was run in duplicate and the pairs that did not agree within 10% of each other were rejected. The values for the DAP activity were expressed as mg of phosphorus (P) liberated by 100 ml of juice. A unit of enzyme activity is defined as the amount of enzyme required to catalyze the liberation of 1 mg of inorganic phosphate in 1 hour under the standard assay conditions. The pH of the duodenal juice (7.8 to 8) which was measured with pHydron paper was the most reliable guide to the position of the catheter tip which was also followed under fluoroscopy.

RESULTS

The mean value for DAP activity of 100 ml of duodenal juice in the group of 10 control children was 4.119 ± 4.32 U (S.D. of 1.371) and in the 17 rachitic children 4.057 ± 2.91 U (S.D. of 1.202). The difference between these means was not significant ($p > 0.05$) and the individual values are indicated in Fig. 1.

COMMENTS

Alkaline phosphatase of serum is heterogenous and comprises activities derived mainly from bone and to a variable extent from the liver and intestine (5, 9). The isoenzymes produced

BAY II 5097 A NEW ORALLY APPLICABLE ANTIFUNGAL SUBSTANCE WITH BROADSPECTRUM ACTIVITY

Preliminary Clinical and Laboratory Experiences in Children

WALTER MARGET and DIETER ADAM

From the Children's Hospital University of Munich Germany

The antimycotic BAY b 5097 bis phenyl (2-chlorophenyl) 1 imidazolylmethane was first described in the summer of 1969 (1 2 3). Animal experiments as well as previous experience with one adult prompted us to start treatment with BAY b 5097 in an infant suffering from a life threatening *Candida* pyelonephritis and then to use it also in other children.

We are observing an increasing incidence of infections with *Candida albicans* during broad spectrum chemotherapy among our patients and therefore urgently need a new drug to treat these children. Subsequent to the combined use of Gentamicin with Carbenicillin or the Cephalosporines in infections of newborns as well as in children with suppression of the immune response induced by diseases or drugs systemic mycoses are increasing in our hospital. Thus during the past year altogether 12 severe systemic mycoses were observed some of which seemed particularly suited for treatment with BAY b 5097 because of the clinically demonstrated unjustifiable side-effects of Amphotericin B (very poor general condition with retention of urea N). An attempt was also made to treat a case of toxoplasmosis with an unfavourable prognosis.

MATERIAL AND METHODS

Clinical studies

The patients were 10 children aged from 5 months to 7 years (Table 1). All children suffered from se-

vere chronic diseases (chronic pyelonephritis with anomalies of the urinary tract, vitæ cordis atopia dermatitis). Besides antibacterial chemotherapy Amphotericin B had been tried previously in 2 children with pyelonephritis and Nystatin locally in the atopic dermatitis. All children had cross infections of *Candida albicans*. No isolated strain was resistant to BAY b 5097 (personal communication of M. Plempe). In some children we were able to find an increased antibody titre to the homologous strain of *Candida albicans* which otherwise is not demonstrable in infants. Those urinary findings which showed more than 10^4 micro-organisms/ml and a simultaneous increase in leucocytes above $50/\mu\text{l}$ were considered as *Candida albicans* pyelonephritis.

The 6 cases of pyelonephritis were treated with a dosage of 70 to 100 mg/kg/day. The treatment lasted for 4 to 40 days.

Laboratory studies

The results of the serum and urine concentration found are based on thin-layer-chromatographic studies. The substance can be isolated with organic solvents from urine, blood or serum and can be prepared in the following manner. Serum is precipitated twice with the same quantity of petroleum ether; the petroleum ether is separated and concentrated for drying. The residue is put in chloroform and is ascendingly chromatographed on silica gel foils with chloroform as a solvent. The dry chromatogram is sprayed with 10% trichloroacetic acid, heated for 2-3 min up to 100°C , the resultant yellow bands are scratched off the foil. 2 ml of butylacetate and 3 ml of perchloric acid are added and measured at 475 nm in the photometer. A $10\text{ }\mu\text{g}$ solution of the preparation is used as reference. This is a method which mainly determines the metabolites of the substance. By means of the agar-diffusion test with stainless steel cylinders according to the recommendations of Shadomy (4) for the testing of Amphotericin B and Flucan, our *Candida* strain can be used to demonstrate the biologically active courses of the blood concentration of BAY II 5097. However we

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Key words Alkaline phosphatase duodenal juice rickets

BAY II 5097, A NEW ORALLY APPLICABLE ANTIFUNGAL SUBSTANCE WITH BROADSPECTRUM ACTIVITY

Preliminary Clinical and Laboratory Experiences in Children

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From the Children's Hospital University of Munich Germany

The antimycotic BAY b 5097 bis phenyl (2-chlorophenyl) 1 imidazolylmethane was first described in the summer of 1969 (1 2 3). Animal experiments as well as previous experience with one adult prompted us to start treatment with BAY b 5097 in an infant suffering from a life threatening *Candida* pyelonephritis and then to use it also in other children.

We are observing an increasing incidence of infections with *Candida albicans* during broad spectrum chemotherapy among our patients and therefore urgently need a new drug to treat these children. Subsequent to the combined use of Gentamicin with Carbenicillin or the Cephalosporins in infections of newborns as well as in children with suppression of the immune response induced by diseases or drugs systemic mycoses are increasing in our hospital. Thus during the past year altogether 12 severe systemic mycoses were observed some of which seemed particularly suited for treatment with BAY b 5097 because of the clinically demonstrated unjustifiable side-effects of Amphotericin B (very poor general condition with retention of urea N). An attempt was also made to treat a case of toxoplasmosis with an unfavourable prognosis.

MATERIAL AND METHODS

Clinical studies

The patients were 10 children aged from 5 months to 7 years (Table 1). All children suffered from se-

vere chronic diseases (chronic pyelonephritis with anomalies of the urinary tract, vitiae cordis atypical dermatitis). Besides antibacterial chemotherapy Amphotericin B had been tried previously in 2 children with pyelonephritis and Nystatin locally in the atypical dermatitis. All children had cross-infections of *Candida albicans*. No isolated strain was resistant to BAY b 5097 (personal communication of M. Plempe). In some children we were able to find an increased antibody titre to the homologous strain of *Candida albicans* which otherwise is not demonstrable in infants. Those urinary findings which showed more than 10^4 micro-organisms/ml and a simultaneous increase in leucocytes above $50/\mu\text{l}$ were considered as *Candida albicans* pyelonephritis.

The 6 cases of pyelonephritis were treated with a dosage of 70 to 100 mg/kg/day. The treatment lasted for 4 to 40 days.

Laboratory studies

The results of the serum and urine concentration found are based on thinlayer chromatographic studies. The substance can be isolated with organic solvents from urine, blood or serum and can be prepared in the following manner: Serum is precipitated twice with the same quantity of petroleum ether, the petroleum ether is separated and concentrated for drying. The residue is put in chloroform and is ascendingly chromatographed on silica gel foils with chloroform as a solvent. The dry chromatogram is sprayed with 10% trichloroacetic acid, heated for 3 min up to 100°C, the resultant yellow bands are scratched off the foil. 2 ml of butylacetate and 3 ml of perchloric acid are added and measured at 475 nm in the photometer. A 10 µg solution of the preparation is used as reference. This is a method which mainly determines the metabolites of the substance. By means of the agar-diffusion test with stainless steel cylinders, according to the recommendations of Shadomy (4) for the testing of Amphotericin B and Flucytosine, our *Candida* strain can be used to demonstrate the biologically active courses of the blood concentration of BAY b 5097. However, we

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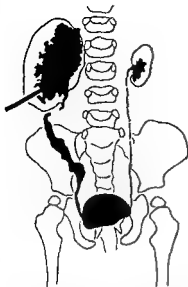


Fig 1 Drawing of a superprojected iv pyelogram and a mictioncystourethrogram of patient 1. Right atresia of the ureter and nephrostomy. Left kidney atrophy.

have not yet gathered sufficient experience to make reliable pharmacokinetic statements for children.

RESULTS AND DISCUSSION

In our opinion it is the child P R which deserves special attention. He was treated in our hospital for the first time when he was in a hopeless condition (Figs 1 and 2). This child showed on the right side an ureterostoma after a hydronephrosis and an obstructive pyelo-

nephritis. After development of an ureterostoma the ureter became completely obstructed. On the left side the iv pyelogram showed an abnormally small kidney. The mictioncystourethrogram on the right side showed a reflux into a strongly dilated ureterstump and on the left normal conditions without reflux. On the basis of this finding we were able to obtain urine separately from the right and the left kidney and to assess the germ counts per ml separately.

In the beginning we did not know the compatibility of this new product in children and therefore we treated at first for 4 days, i.e. as long as *Candida albicans* had been eliminated from the urine almost completely. A relapse occurred after 22 days whereupon the drug was given in the same dosage for 11 days. Again after discontinuation a renewed life threatening relapse occurred which could be controlled after a duration of treatment of 18 days with the same dosage and which led to the healing of the *Candida* infection. In the subsequent period (2 years) the child had no further relapse and thrived normally.

The serum concentrations in 2 patients are shown in Table 2. In the first hour it was 11 in the third hour 10–20 and in the sixth hour 13–25 $\mu\text{g/ml}$ 10 hours after administration a clearcut decrease could be observed except in the child with burns on the seventh day of treatment. Excretion was relatively slight. Thus a 12 hours excretion of 10–15 mg or 7–05

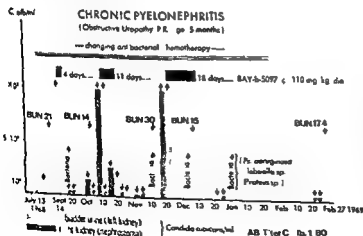


Fig 2 *Candida albicans* pyelonephritis after various periods of treatment with BAY b 5097 in patient 1. Relapse after 4 days treatment, after 11 days treatment and 4 weeks interval with out treatment a serious recurrence with increase in BUN. Healing after 18 days of treatment.

Table 1 Clinical summaries of ten patients treated with BAY b 5097

Name Patient no	Age (years)	Diagnosis	Diagnostic findings			Therapy			Results after treatments			Relapse	Notes
			Infection due by C albicans	Number of organisms in urine/ml ^a	WBC in urine per µl	Blood culture	BAY b 5097 mg/kg/day	Days	Candida albicans findings in urine	Side Effects			
P ^b 1	1	Obstructive uropathy	Chronic pyelonephritis (PN)	>10 ⁴	250	Neg	110-140 (2 × 80)	4/11/18	Neg some organ	None	44 ^c see fig 1a 2		Candida albicans hemaggl AB titre 1 100 After dis continuing normalized Transaminases
F ^c 2	4	Deep scalding 70 (grade II)	PN	>10 ⁴	160	Neg	120 (2 × 60)	9	Neg	SGOT 59.3 SGPT 40.8 Alk Phos 157.40	No 30 ^c		Further treatment
S ^c 3	10/12	Meningo myelocle	Chronic PN	5 × 10 ⁴	500-1 000	—	70	21	Neg	Weariness	—		Further treatment
W ^c 4	1 6/12	Transv lesion of the cord	PN	>10 ⁴	80	—	100 (2 × 50)	17	Neg	None	No 5 ^c		(Pat out of control)
M ^c 5	3 7/12	Hydropy nephrosis	PN	10 ⁴	500	—	75	10	Neg	None	No 7		5 weeks after discontinuing relapse
H ^c 6	5	Atopic dermatitis	Candidiasis with positive feces and throat swab	—	—	Neg	100 (2 × 50)	38	—	None	Yes		
G ^c 7	2 9/12	Hydropy nephrosis	PN	>10 ⁴	140	—	70	21	Neg	None	No 14 ^c		
H ^c 8	9 9/12	Meningoence phalitis (Tbc)	Candida septicemia	—	—	+++	75-100	6	—	None	—		Exitus (Tbc) blood cult
R ^c 9	4	Ventricular septum defect	Candida septicemia	—	—	+++	135 (3 × 45)	12	—	None	—		Candida neg Further treatment
H ^c 10	11/12	Severe congenital Toxoplasmosis 2 relapses after Daraprim and Sulfonamides	—	—	—	—	150-250 ^a	40 ^a	—	(Slight weariness)	No 22 ^c		Sabin s dye test before 1 4 000 after treatment 1 4 000 KBR before 1 20 after treatment 1 5

Findings refer only to Candida albicans and not to other causative organisms of chronic pyelonephritis (Candida pyelonephritis as a mono-infection was never observed)

^a Compare Figs 1 and 2^c Observed period after the end of treatment in weeks



Fig 1 Drawing of a superprojected i.v. pyelogram and a mictocystourethrogram of patient 1. Right atresia of the ureter and nephrostomy. Left kidney atrophy.

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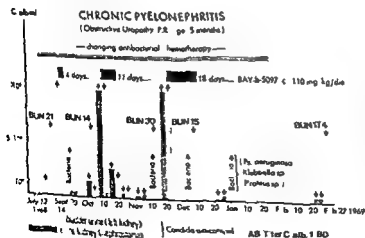


Fig 2 *Candida albicans* pyelonephritis after various periods of treatment with BAY b 5097 in patient 1. Relapse after 4 days treatment, after 11 days treatment and 4 weeks interval without treatment a serious recurrence with increase in BUN. Healing after 18 days of treatment.

Table 2 Concentrations of BAY b 5097 (Metabolite) in the serum of patients 6 and 2

Time after administration (h)	Case 6		Case 2	
	Onset of therapy (1st day) twice 50 mg/kg/day ($\mu\text{g/ml}$)	Cont therapy (4th day) twice 50 mg/kg/day ($\mu\text{g/ml}$)	Onset of therapy (1st day) once 60 mg/kg/day ($\mu\text{g/ml}$)	Cont therapy (7th day) twice 60 mg/kg/day ($\mu\text{g/ml}$)
1	11			
3	17	10	11	20
4	18			
5		14		
6	16		13	25
7		13		
9		8		
10	14		6	24
11		6		
13		5		11
24			6	

mg could be determined in case 6, on the first to fourth day with a total ingestion of 1 g of the substance

In case 2, also receiving a total daily amount of 1 g a 24 hours' excretion of only 24 mg could be found. As shown in Table 1, no relapses were observed after treatment of pyelonephritis had been discontinued, though the substance used for the treatment was a drug having a fungistatic effect only. Furthermore, we treated a toxoplasmosis in a hopeless condition. Previously, the child had been treated at an interval of several weeks twice with Daraprim and Sulfadiazine in a high dosage for 3 weeks. After long term treatment with BAY b 5097, during which we administered up to 200 mg/kg daily, a probable healing could be observed which persisted until the time of writing (22 weeks).

In children with severe chronic diseases displaying, due to their disease, an increased disposition to the demonstrated infection with *Candida albicans* we were able to carry out successful treatment with BAY b 5097. This leads to the conclusion that this substance has a high therapeutic effect in view of the susceptibility of the patients. Blood concentrations of the metabolites show that the product is very well absorbed and shows a retarded excretion. According to our studies it is still not

clear how the drug is excreted, whether via the intestine or the bile or another route. We were unable to demonstrate considerable amounts of the substance or its metabolites in the urine.

In order better to evaluate the drug, further follow up of the clinical therapeutic results is necessary. We must also understand the pharmacokinetics of biologically active substances and determine the mode of excretion. The efficacy of the drug in infections with *Candida* in severely predamaged children is remarkable, particularly in view of its fungistatic action.

Side-effects

In one child with a 70% second degree burn, showing renal damage and pyelonephritis we could observe a cumulative effect of the product (Table 2 case 2). This concentration led to a remarkable fatigue and an increase of the transaminases SGPT and SGOT. These symptoms disappeared immediately after discontinuation of the drug.

SUMMARY

BAY b 5097 bis phenyl (2-chlorophenyl) 1-imidazolylmethane, is a fungistatically acting new antimycotic which was used in 10 chil-

dren in a dosage of 70–200 mg/kg/day usually in two doses. Six children suffered from a *Candida albicans* pyelonephritis, 2 from *Candida* septicemia, 1 from atopic dermatitis with candidiasis and 1 from congenital toxoplasmosis. All children responded to the therapy. The possible successful treatment of a hopeless case of toxoplasmosis leads to the assumption that there might be an effect on protozoa.

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CASE REPORT

THE CUTIS VERTICIS GYRATA AND MENTAL RETARDATION SYNDROME IN A 4-YEAR OLD BOY

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The combination of cutis verticis gyrata (corrugated or gyrate appearance of the scalp) and mental retardation has been published in more than one hundred cases. A maldevelopment of skull and/or brain is encountered in most and probably all patients (4). Certain evidence points also to an endocrine disorder (1, 2).

Very few female patients with the cutis verticis gyrata and mental retardation syndrome have been reported. In addition, the scalp anomaly has been observed in patients who have reached or already passed their puberty. The only exception was an 11 year old girl (5). The rare onset before puberty and the disproportion between the sexes are the most widely quoted criteria to support the theory that cutis verticis gyrata is caused by an endocrine disorder. We would like to present a 4-year old boy with cutis verticis gyrata and mental retardation. He is the youngest patient ever seen with this rare condition.

CASE REPORT

The patient was born August 30, 1966. The father, mother, two siblings (born in 1960 and 1964) and all other members of the family were healthy. The pregnancy was normal. The delivery was 3 weeks over due and labour was induced. The birth weight was 3980 g and length 51 cm. The patient scored 10 points in the Apgar scale. The head circumference

was small, 33.5 cm, and the parietal bones were overlapping in the sagittal suture, but this was within the normal limits. At the age of 1 month it was observed that the head circumference did not increase and at the age of 6 weeks the child had his first convulsions. At 3 months he had status epilepticus and was hospitalized. The EEG recording consisted of high and slow (2-6 c/s) waves mixed with frequent spikes and sharp waves. The finding was somewhat typical for hypsarrhythmia, although the very high number of spikes and the localization of the abnormal waves to the right side did not quite correlate to the diagnosis. The convulsions were also different from infantile spasms. Other clinical findings were microcephaly, spastic tetraplegia and unspecific generalized aminoaciduria. The scalp was normal except for dandruff and erosions at the age of 5 months. The patient was unresponsive to visual and auditory stimuli. A treatment with ACTH was started and given during 6 weeks at the age of 5-6 months. During that period only a few mild convulsions were seen but afterwards the patient had a typical Cushing face. The EEG showed no hypsarrhythmia but there were some bursts of sharp and slow waves more on the left. He was then on a constant anti-epileptic medication but had nevertheless occasional grand mal seizures. The cutis verticis gyrata was observed in connection with a cutaneous infection of the scalp.

At the age of 3 years and 2 months the patient was unable to sit or walk. The head was asymmetric with a circumference of 39.5 cm. Four folds were easily palpable and visible in the left parietal region of the scalp (Fig. 1). Micrognathia, short neck and marmorated skin were also observed. The patient was probably blind with a horizontal bilateral continuous nystagmus and an outward deviation of the left eye. He seemed to react to strong sounds. The marked loss of hearing ability was confirmed with the evoked



Fig 1 Four cutis verticis gyrata folds are visible on the left parietal area of the scalp



Fig 3 Gas encephalography showing enlarged ambient cisterns. The brain stem is hypoplastic. Note the markedly asymmetric skull.

potential examination. He had thoracic scoliosis and mild contractures in both knees. All the extremities showed some spasticity during active movements although they felt quite hypotonic during rest. Babinski sign was positive on both sides. The patient reacted to pain. He had no bladder or bowel control and could not eat or clothe himself. His IQ was well below 10. X-ray showed asymmetric small cranium where the left side was larger than the right side. A marked asymmetric macroventriculopathy (Fig. 2) was observed in pneumoencephalography together with a hypoplastic brain stem (Fig. 3) and cerebellum (Fig. 4). The width of the third ventricle was 16 mm.

Routine blood, urine and cerebrospinal fluid tests showed no specific changes. Chromosome analysis was normal. An ACTH loading test was also performed. Serum cortisol value was $42.0 \mu\text{g}/100 \text{ ml}$ before the intramuscular injection of 20 IU of natural ACTH (Cortrophine®). $77.7 \mu\text{g}/100 \text{ ml}$ after 1 h, $75 \mu\text{g}/100 \text{ ml}$ after 2 h, and $53.1 \mu\text{g}/100 \text{ ml}$ after 3 h of the injection. Thorn's test confirmed the normal adrenocortical function (31 blood eosinophils/ mm^3 before the test and $6/\text{mm}^3$ after 4 h of the intramuscular injection of 20 IU of natural ACTH).

DISCUSSION

Although maldevelopment of skull and brain is very frequent in the cutis verticis gyrata and



Fig 2 Gas encephalography showing asymmetric, markedly enlarged lateral ventricles. The frontal horns and cella media of the left lateral ventricle are larger than those of the right one.



Fig 4 Gas encephalography showing hypoplastic cerebellum and brain stem in lateral projection.

mental retardation syndrome (4), the origin of the scalp malformation remains obscure. Endocrinological factors may have some etiological significance, this hypothesis is supported by the fact that most patients are males and that only 2 paediatric cases have thus far been diagnosed. The present patient was given ACTH in early infancy. It can be speculated that this treatment either caused cutis verticis gyrata or at least had some contributory effect to its formation. However, there were no known cases of cutis verticis gyrata among 70 children treated with ACTH because of infantile spasms at the Children's Hospital in Helsinki. Congenital existence of the malformation is, of course, more obvious. The scalp may become more easily folded, when the skull has a small circumference (3). The encephalographic findings and the existence of several malformations support the theory of prenatal origin of the disorder, and thus the hypothesis of the secondary nature of possible endocrinological abnormalities. At present the patient seems to have a normal pituitary and adrenocortical function despite of the wide third ventricle. This is in agreement with the results obtained in a larger series where no consistent endocrinological abnormalities could be observed (4).

The 4 year-old boy with the cutis verticis gyrata and mental retardation syndrome described above is an interesting example of a syndrome that was long believed to appear only after puberty. The development of the scalp anomaly will be followed.

SUMMARY

A 4 year old profoundly mentally retarded boy with cutis verticis gyrata is described. He had four folds on the left parietal region, small

asymmetric head, micrognathia, short neck, marmorated skin, thoracic scoliosis, spastic tetraplegia and epilepsy. Encephalography revealed marked asymmetric macroventricles with hypoplastic brain stem and cerebellum. Thorn's test and corticotrophin stimulation test gave normal responses. The boy is the youngest patient with the cutis verticis gyrata and mental retardation syndrome described in the literature. The numerous malformations of the patient strongly support the theory of prenatal origin of the cutis verticis gyrata and mental retardation syndrome.

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Key words: Child, cutis verticis gyrata, mental retardation.

CASE REPORT

A CASE OF TUBEROSE SCLEROSIS IN THE NEWBORN

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The first record of sclerosis of the cerebral cortex accompanied by myomata of the heart was made by Von Recklinghausen in 1862 and later by H Vogt (4) The clinico-pathological syndrome which we know today as the tuberose sclerosis complex has been summarised by H Globus (3) in recent times

This consists of an association of cerebral sclerosis with hamartomata of the skin heart, kidney bone and lung and is clinically recognised by the association of adenoma sebaceum and epilepsy The cardiac hamartomatous lesions are usually called rhabdomyomata which are commonly present as multiple nodules throughout the substance of the heart

Batchelor & Mann (2) showed that at least 50% of cases of cardiac rhabdomyoma were associated with cerebral sclerosis whilst about 7% of those with cardiac lesions died in the neonatal period

CASE REPORT

The mother of the affected infant was a 25 year-old primigravida, induced and delivered by forceps of a live male infant weighing 2346 g under the care of Dr B B Corner The onset of inspiration was spontaneous but then became irregular and gasping. Intubation and intermittent positive pressure respiration failed to maintain adequate oxygenation the cardiac rate slowed and a harsh pansystolic murmur was heard The infant died aged 1 hours.

Post mortem findings

The body was that of a mature cyanosed male infant of 43 cm crown-heel length. The right pleural cavity contained gas under pressure indicating the presence of a tension pneumothorax The pericardium contained an excess of clear serous fluid.

The heart was grossly distorted by the presence of several tumour masses within its substance, the largest being 6 cm in diameter in the intraventricular septum There was another smaller lesion 1.5 cm in diameter situated just below the valve (Fig 1) Many other small nodules were present throughout the ventricular myocardium (Figs 1 and 2) Both lungs were small and the left lung was completely unexpanded whilst expansion was only partial within the right The kidneys showed a few small cysts measuring up to 0.4 cm in diameter Other organs throughout the body appeared to be normal

Histology

Sections of the renal cysts showed them to be lined by a rather hypertrophic tubular type of epithelium

Sections from the cardiac tumours were fairly typical of the so-called "Rhabdomyoma" consisting of a loose network of thin striated muscle grossly vacuolated by glycogen deposits to give an open mesh appearance within the cytoplasm of the swollen myocardial cells (Fig 2)

Central nervous system

At first, examination of the brain showed no appreciable abnormality but after fixation although the gyral pattern was normal many large irregular rubbery tubers could be seen and palpated in the gyri of both cerebral hemispheres Coronal slicing of the specimen revealed irregular thickening and distortion of the cortical ribbon wherever a tumour was present There were also numerous firm pale nodules seen throughout the central white matter of each hemisphere (Fig 3) Similar nodules were also seen beneath the ependyma of the lateral ventricles some of these projected into its cavity especially into that of the anterior horn on the right side.

No heterotopic nodules could be recognised within the brainstem or within the substance of the cerebellum Sections were taken from numerous areas of the brain including the cerebral cortex through to the lateral ventricles, the basal grey nuclei the brainstem and cerebellum

Histologically many of the typical neuropathological features of tuberose sclerosis were present The tubers within the cortex consisted of aggregates of grossly

mental retardation syndrome (4), the origin of the scalp malformation remains obscure. Endocrinological factors may have some etiological significance; this hypothesis is supported by the fact that most patients are males and that only 2 paediatric cases have thus far been diagnosed. The present patient was given ACTH in early infancy. It can be speculated that this treatment either caused cutis verticis gyrata or at least had some contributory effect to its formation. However, there were no known cases of cutis verticis gyrata among 70 children treated with ACTH because of infantile spasms at the Children's Hospital in Helsinki. Congenital existence of the malformation is, of course, more obvious. The scalp may become more easily folded, when the skull has a small circumference (3). The encephalographic findings and the existence of several malformations support the theory of prenatal origin of the disorder and thus the hypothesis of the secondary nature of possible endocrinological abnormalities. At present the patient seems to have a normal pituitary and adrenocortical function despite of the wide third ventricle. This is in agreement with the results obtained in a larger series where no consistent endocrinological abnormalities could be observed (4).

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Key words: Child, cutis verticis gyrata, mental retardation.



Fig. 3 Coronal slice showing tubers in cortex and subependymal nodules.

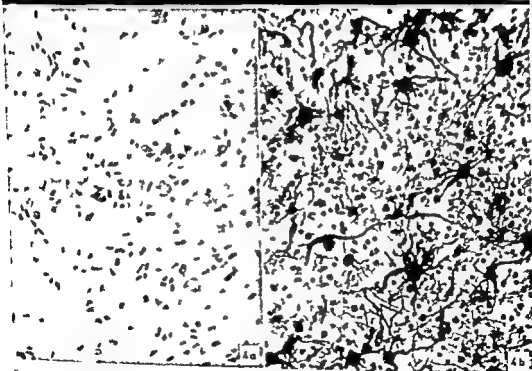


Fig. 4 (a) Heterotopic nodule of giant astrocytes. H&E, 100 (b) A similar area after silver impregnation SCA, 100

to normal astrocytes causing disturbance of lamination but not associated with any excess of glial fibre production. Many of the cells constituting these tubers

were bi- or tri-nucleate as were similar collections of cells within the substance of the white matter which were partly astrocytic and partly neuronal (11).

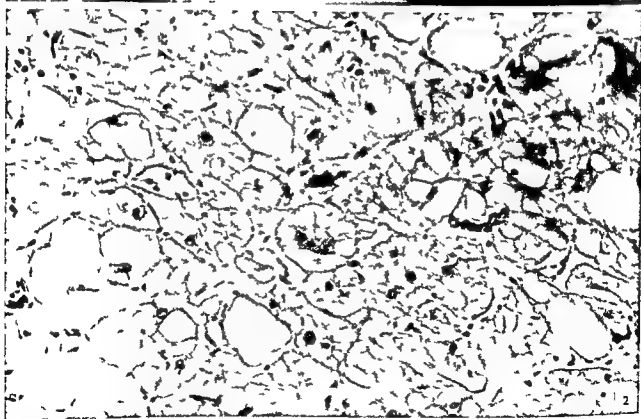


Fig 1 Tumour obstructing pulmonary outflow tract

Fig 2 Rhabdomyoma showing vacuolated cardiac muscle cells H&E $\times 200$



Fig 3 Coronal slice showing tubers in cortex and subependyma nodules

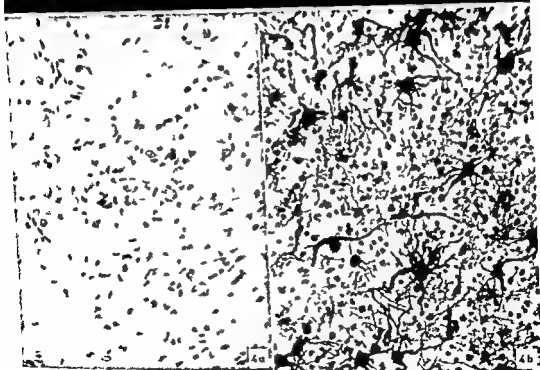


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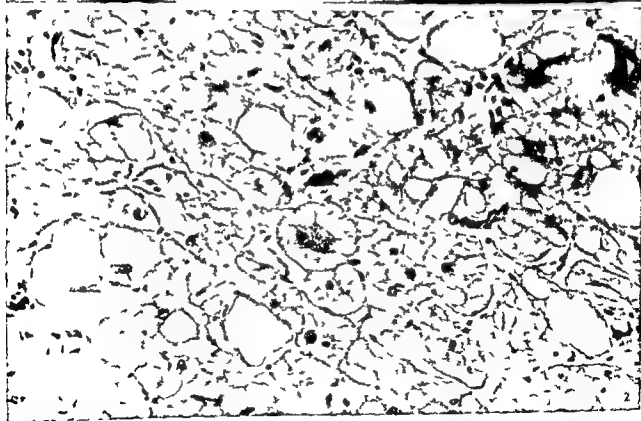


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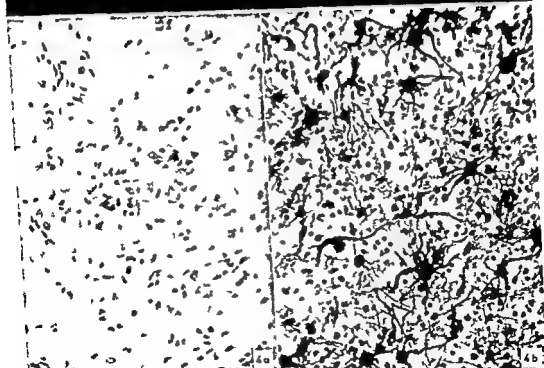


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Fig 5 Fibroblasts and glial cells in a subependymal nodule PTAH $\times 100$

nature (Fig 4). The subependymal nodules were of the typical fibro cellular type consisting of large round or oval cells intermingled with tufts of glial fibrils originating from the interspersed fibrillary astrocytes (Fig 5). No heterotopic nodules could be seen in the brainstem or cerebellum; neither were there any giant Purkinje cells demonstrated within the cerebellar cortex.

DISCUSSION

This infant presented as a resuscitation problem, the immediate cause of death being disturbance of cardiac function due to obstruction of the pulmonary outflow tract by the presence of the numerous and large cardiac tumours. Because of the typical lesions in the brain and the rhabdomyomata of the myocardium this infant must be considered as a random dominant case of Tuberose sclerosis. In this case there is no family history of any similar disorder.

The extent of the lesions in the brain was also unusual and presumably indicates a more complete expression of this variable disorder of histogenesis (Alzheimer (1)) than is usually seen.

The heavy gliosis, normally seen in this condition was not seen in this case, but death occurred early in the neonatal period and reactive

changes would not have had time to occur. The little gliosis that was seen could well have occurred during intrauterine life.

SUMMARY

This paper describes an infant with tuberose sclerosis which died in the neonatal period from cardiac failure associated with multiple Rhabdomyomata of the heart.

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CASE REPORT

ECTOPIC SPLEEN IN AN Rh INCOMPATIBLE INFANT

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Ectopic spleen is a rare condition in the newborn period. The diagnosis is to be considered in the presence of a tumoral mass in the abdomen of the newborn in the absence of a detectable spleen at its usual site. This condition has to be kept in mind especially when an abdominal mass is present in a newborn with Rh incompatibility where splenomegaly is expected but no spleen is palpable at its normal position.

The following is a case in which an ectopic spleen was diagnosed preoperatively in a newborn in the presence of Rh incompatibility with a relatively high level of conjugated bilirubin.

CASE REPORT

The mother was a 38 years-old Jewish woman of Iraqi origin. Her present pregnancy was the seventh and she has given birth to six children. The blood group of the mother was found to be B Rh negative. No signs of hemolytic disease were observed in the first six children although all of them were Rh positive.

The present pregnancy developed normally and the titer of the indirect Coombs test rose to 1:64 2 weeks before the delivery. A male baby of 4000 g was delivered spontaneously in the 40th week of gestation. Three hours after delivery he became pale and a slight degree of jaundice developed. The liver was palpable 4 cm below the costal margin. No spleen was palpable in left upper quadrant. A firm oval mass about 5-6 cm was found in the left lower quadrant (Fig. 1). It had a smooth surface and was freely mov-

able about 2 cm in all directions. No pain seemed to be elicited in the infant on examination of the mass. A provisional diagnosis of Ectopic spleen was made.

Laboratory findings. The total cord blood bilirubin was 4.5 mg/100 ml from which the direct binding fraction represented 1.5 mg/100 ml. Hemoglobin 12.0 g/100 ml. Blood group was found to be B Rh positive and the direct Coombs test was positive. Chest and abdominal X rays showed no pathological findings. 20 hours after birth the hemoglobin was found to be 11 g/100 ml. The leukocyte count was 34 700/mm with 40% normoblasts and the reticulocyte count was 13%. The bilirubin rose to 11 mg/100 ml with 3 mg/100 ml direct binding bilirubin. The urine was found positive for bilirubin.

On the 5th day the hemoglobin level went down to 9 g/100 ml and the total bilirubin reached 32 mg/100 ml, of which 22 mg/100 ml was of conjugated form. An exchange transfusion was performed mainly to arrest the increasing anemia. 400 ml of B Rh negative blood was used for the exchange. In spite of another rise in the total bilirubin level, a second exchange transfusion was not considered to be necessary since the hemoglobin was stable at about 13 g/100 ml and the indirect bilirubin was found to be only one third of the total. Gradually the jaundice subsided and at 2 weeks of age a slight decrease in the volume of the mass in the left lower quadrant was noted. After an intravenous drip pyelography and barium enema were performed and found normal the possibility of the mass being an ectopic spleen was investigated. A scintigram was performed after injecting ^{51}Cr labeled autologous red blood cells (Fig. 2). This revealed the presence of a round mass about 11 cm diameter in the left abdomen but neither in the position of a normal spleen, nor where the mass was previously palpated but midway between these two points.

Exploratory laparotomy was performed at the age of 6 weeks. An intraperitoneally situated ectopic



Fig 1 Patient after birth without palpable spleen at usual site but with round mass in lower left abdomen

spleen with an infarcted area at its upper pole was found in the left iliac fossa on a long partially torsioned vascular pedicle.

Normal ligaments connecting the spleen to its neighboring organs were not found although the spleen was bound to the ileum by numerous adhesions. The gallbladder and the extrahepatic bile ducts appeared normal and no accessory spleen was seen. Splenectomy was performed in view of the ectopic infarcted spleen, the adhesions and the torsion of its long pedicle.

The ectopic spleen was enlarged, weight 110 g and the infarcted upper part was well delineated from the remainder (Fig 3). At microscopy apart from the large infarcted area the remainder of the splenic tissue was normal with an increased number of normoblasts and an excess of iron pigment.

The post-operative course was uneventful. The baby was discharged a month later with a thrombocyte count of $800\,000/\text{mm}^3$. No serious infections occurred in the infant during a 24 months follow up period.

DISCUSSION

Wandering spleen is a rare condition usually found in patients with splenomegaly and laxity



Fig 2 The scintigram after injection of ^{51}Cr labeled autologous red blood cells revealed a mass midway between the palpated tumor and the usual position of an enlarged spleen

of the abdominal wall. In a series of 1 437 cases of splenic diseases seen over a period of 10 years not a single case of wandering spleen was reported (6). The finding of an ectopic spleen in a newborn infant is an even greater rarity and possibly related to developmental defects of the fixation of the spleen in its nor-



Fig 3 Section through the enlarged spleen with the infarcted area at the upper pole

mal position. Apart from this condition involving the sites of the spleen, varying numbers of "accessory" or supernumerary spleens are much more common. Their most frequent sites are the hilum of the spleen, gastrosplenic ligament, and the greater omentum, and rarely the scrotum, liver and pancreas (2).

The spleen appears in embryos of about 10 mm crown rump length as a condensation of mesodermal cells in the dorsal mesogastrium. The enlarging spleen later projects through the left leaf of the dorsal mesogastrium and the mesentery dorsal to it rotates backwards to the left and fuses with the peritoneum of the posterior abdominal wall. Dorsal to the spleen the mesentery forms the lienorenal ligament and ventral to it the mesentery forms the gastrosplenic ligament (3). It has been suggested that a poorly developed gastrosplenic ligament allows excessive splenic mobility (5). Increased mobility of the splenic hilum may also be due to the incomplete fusion of the mesogastrium posteriorly while at the same time the lienorenal ligaments is not formed (7).

Although the spleen enlarges in fetuses severely affected by Rh incompatibility only in the last third of the pregnancy its increase in weight and volume could at least theoretically influence its location should some earlier developmental defect preexist. In the present case the spleen at operation was found close to the bulk of the ileum with numerous adhesions between them and since no suspensory ligaments of the spleen were found it must have been ectopic.

This hypothesis is supported by the surprising similarities with the case reported by Daum et al. of an erythroblastotic newborn with an enlarged spleen discovered in the right lower abdomen and no suspensory ligaments found at operation (1). The features of the two cases are listed in Table 1.

The finding of a high level of conjugated bilirubin in some cases of erythroblastosis fetalis is known (4). There are no evident links between ectopic spleen and the obstructive type of jaundice observed in our patient. Never-

Table 1 Similarities of features between the present case and the patient reported by Daum and coworkers

Essential data	This case	Daum's case
1 Hemolytic disease due to Rh incompatibility	Present	Present
2 Exchange transfusion performed	Once	Twice
3 Obstructive type of jaundice	Present	Present
4 Spleen at normal site	Absent	Absent
5 Mass in lower abdomen (ectopic spleen)	Present (left)	Present (right)
6 Suspensory ligaments of spleen	Not found	Not found
7 Partial infarction of ectopic spleen	Present	Present

theless it is intriguing that the same association existed in the only additional case of ectopic spleen in erythroblastotic newborn found in the literature.

SUMMARY

A case of a newborn with an Erythroblastosis fetalis and obstructive type jaundice with an abdominal tumor is presented. As the jaundice was found to be elicited by Rh incompatibility the absence of a palpable spleen at the normal site suggested the possibility of the abdominal mass being an ectopic spleen. This diagnosis was confirmed at operation. The possibility of a preexisting developmental defect is elaborated upon.

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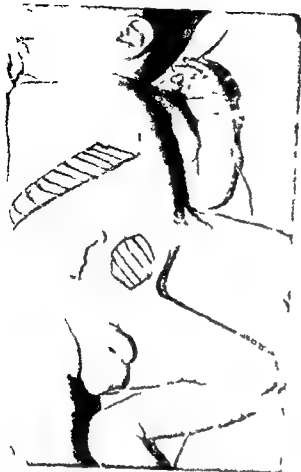


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DISCUSSION

'Wandering' spleen is a rare condition usually found in patients with splenomegaly and laxity

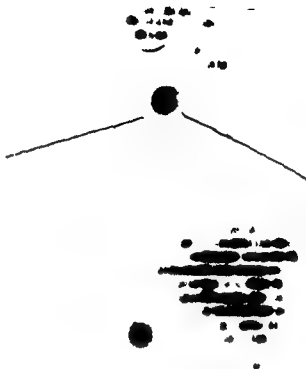


Fig 2 The scintigram after injection of ⁵¹Cr labeled autologous red blood cells revealed a mass midway between the palpated tumor and the usual position of an enlarged spleen

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Fig 3 Section through the enlarged spleen with the infarcted area at the upper pole

CASE REPORT

CUTIS LAXA ASSOCIATED WITH SEVERE INTRAUTERINE GROWTH RETARDATION AND CONGENITAL DISLOCATION OF THE HIP

S H REISNER, M SEELINFREUND and M BEN BASSAT

From the Department of Paediatrics Orthopaedics and Pathology of the Beilinson Hospital Petah Tikva and the Tel Aviv University Medical School Israel

Congenital cutis laxa is a rare familial disorder (7). Characteristically the skin hangs loose in pendulous folds giving the patient an aged appearance. As this is a disease of the connective tissues other organs may also be affected. Therefore apart from the cosmetic disturbance the condition may cause lethal generalised illness depending on the organ affected (9, 10, 12).

The purpose of this paper is to report two siblings with congenital cutis laxa associated with severe intrauterine growth retardation (IUGR) and congenital dislocation of the hip (CDH).

CASE REPORT

Case 1 C N

This infant was the second child born to a 26-year old asthmatic mother and a 32-year-old healthy father. The parents were first cousins. The duration of pregnancy was 47 weeks and the delivery was normal.

Her birth weight was 2150 g, height 43.5 cm and head circumference 32 cm. On examination she was noted to have a very dry, wrinkled skin with large tortuous veins (Fig. 1). The face was wrinkled and the eyes were sunken. The ears were low set. Both fontanelles were large: the anterior approximately 6 cm wide and the posterior 3 cm wide with the sutures markedly separated. The palate was high arched. A transverse palmar crease was noted on the right hand. On examination of the hips, a "click" was elicited in the left hip joint and CDH was

confirmed by X-ray. Treatment with conservative measures was unsuccessful.

At the age of 1 year she weighed 6300 g (50th percentile for 4 months), her height was 68 cm (50th percentile for 6 months). She spoke a few words and her responses were within normal limits for her age. A marked redundancy of skin was noted particularly of the abdomen, hands and feet, with distended veins. Hyperlaxity of all the joints was found.

Blood and urine examinations at the age of 1 year were all normal except that on electrophoretic fractionation of the serum an elevated level of alpha 2 globulin was found. Cytogenetic studies showed a normal female karyotype.

At the age of 14 months open reduction of the left hip with limbectomy was successfully performed and a skin biopsy was taken.

Pathological report (Fig. 2). On histological examination of the skin using Weigert's elastic stain the elastic fibres were noted to be decreased in amount especially in the papillary region with degenerative changes. Some of the fibres were fragmented, others were either shorter or longer as well as thinner than normal fibres. Control sections of the skin of the thigh of several healthy infants showed none of these changes.

The pathological changes were compatible with the diagnosis of cutis laxa.

Case 2 C T

This is the older sister of case 1. She was born following an otherwise normal pregnancy of 36 weeks duration weighing 1400 g. At the age of 4 months the infant was referred to the orthopaedic department for a suspected right CDH which was confirmed by X-ray. She was treated with conservative measures without any improvement. At the age of 1 1/2 years, open reduction of the right hip with limbectomy

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Key words Ectopic spleen Rh incompatibility newborn

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Fig 1 Patient CN at the age of 2 days showing wrinkled skin with dilated veins on abdomen

and derotation osteotomy of the femur were successfully performed

She was first examined in the paediatric clinic at the age of 4 years. Her weight was 11 700 g (50th percentile for 2 years), her height was 97 cm (10th percentile) and her head circumference was 45 cm (50th percentile for 1 year). There was marked redundancy of the skin with distended veins. She had increased amounts of hair over her entire body. Hypertelorism with bilateral epicanthus was noted. Her palate was high and the ears were low set. Hyperextensibility of the joints was found especially of the knee joints. The wound of the operation on the right hip had healed. Her development was slow in all respects.

On ophthalmological examination coloboma of the macula was found in both eyes as well as severe myopia.

DISCUSSION

The clinical diagnosis of congenital cutis laxa (chalazoderma, dermatomegaly, dermatolysis

or primary elastolysis) can usually be made by the typical skin findings. The loose pendulous skin gives the affected person an aged appearance and in children it is suggestive of a suit of skin too large for the owner. The disease is rare. Goltz et al (7) reviewed the literature until 1965. They found 19 cases and added 2 of their own. Subsequently a further 10 infants with this disorder have been reported (1, 2, 3, 5, 9, 10, 11, 12). In most of the cases reported the birth weight of the infants was normal. It appears to be more common in females than in males. Involvement of connective tissues of internal organs may result in pulmonary emphysema with right heart disease (7, 9, 12), gastrointestinal and urinary tract diverticulosis, vaginal and rectal prolapse, and the occurrence of a deep voice (7). Other findings include pulmonary artery stenosis (10), degenerative changes in the cornea (2), with severe mental retardation (3). In contrast to the Ehlers Danlos Syndrome where hyperlaxity of the ligaments with instability of the joints are usual features in cutis laxa no hyperextensibility is usually found (8), although this was noted in an infant reported by Debre (4).

In 1942 Fittke (6) reported a female infant born at term weighing 2 275 g with a birth length of 46 cm (Table 1). No consanguinity was known. At birth the skin was noted to be redundant. At the age of 10 1/2 months the diagnosis of cutis laxa with right CDH was made.



Fig 2 Skin biopsy in patient CN. The amount of elastic tissue is decreased in the upper dermis. $\times 400$

Table 1 Clinical findings in reported patients of cutis laxa with IUGR and CDH

Case	Sex	Period of gestation	Birth weight (g)	Birth length (cm)	Consanguinity	CDH	Hyperlaxity
Fittle 1942 (6)	F	Term	2 275	46	None known	Rt hip	?
Bittel Dobrzynska 1964 (1)							
Case 1	F	Term	2 500	—	None	Bilateral	Yes
Case 2	F	Term	2 000	44	None	Bilateral	Yes
Case 3 (Sibling of 2)	F	Term	1 750	43	None	Bilateral	?
Reisner et al.							
Case 1	F	42 weeks	2 150	43.5	Yes	Lt hip	Yes
Case 2 (Sibling of 1)	F	36 weeks	1 400	—	Yes	Rt hip	Yes

No similar cases were reported until 1964 when Bittel Dobrzynska & Simecki (1) reported 3 cases. The pertinent details are given in Table 1.

These 4 cases and our 2 infants have a number of findings in common (Table 1). They are all female infants born after a full term or post term pregnancy except for our case 2. All their birth weights were usually far below the 10th percentile for their period of gestation. In those infants in whom the birth length was known this was also under the 10th percentile for gestation. All had congenital dislocation of either one or both hips and in at least four of the six hyperlaxity of some joints was mentioned.

These 6 infants appear to present a syndrome different from all the other reported cases of cutis laxa. They are all female patients with IUGR both in weight and length. Apart from the typical skin manifestations they present with hyperextensibility of their joints associated with CDH. The mode of inheritance appears to be as an autosomal recessive gene. The marked degree of IUGR may be indicative of a more severe form of the disease which might be lethal in the male foetus.

SUMMARY

Two female siblings with cutis laxa are reported. Both these infants showed marked intrauterine growth retardation, hyperlaxity of

the joints with congenital dislocation of the hip. Four similar female infants have been reported in the literature. The mode of inheritance appears to be as an autosomal recessive gene. The marked degree of intrauterine growth retardation may be indicative of a more severe form of the disease which might be lethal in the male foetus.

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- Key words** Cutis laxa intrauterine growth retardation congenital dislocation of the hip

LETTER TO THE EDITOR

To the Editor

Recently an article by O Seriki on the inheritance of osteogenesis imperfecta congenita was published in your journal (*Case Report Osteogenesis Imperfecta Congenita in One of Twins* O Seriki *Acta Paediat Scand* 59 340-342 May 1970) Two cases were reported each concerning a set of twins in which one twin was afflicted with the disease and the other twin was not In each case only one of the parents was affected (the mother in Case 1 the father in Case 2) Although the twins were like sexed in both cases investigations to determine zygosity were not carried out in view of the fact that in both cases the twins were markedly dissimilar in appearance it does not seem feasible to propose monozygosity merely on the basis of sex.

Osteogenesis imperfecta congenita is generally considered to be inherited as an autosomal dominant trait with considerable variation in degree of expressivity The author of the twin studies suggests that the affected parents (mother in Case 1 father in Case 2) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic Assuming that the affected parents were heterozygous carriers the other parent in each case was reported to be normal In such a case it seems unlikely for the condition to be transmitted as a recessive but rather it appears to be a case of autosomal dominant inheritance

Considering the lack of information regarding the parental genotype and the absence of any zygosity investigations I find it impossible to suggest transmission as a recessive characteristic

Patrick Sweeney
Senior Medical Student
St. Louis University School of Medicine
St. Louis Missouri

The Editor has asked Dr Seriki to comment on this letter

To the Editor

Mr Sweeney raises three points namely that (1) investigations were not carried out (2) it does not seem feasible to propose monozygosity merely on the basis of sex and (3) that mode of inheritance is one of autosomal dominant.

In reply to the above criticism I would state that investigations were carried out but were incomplete because of sudden death in one twin of each pair For this reason, it was not possible to establish zygosity and they were labelled like sexed twins instead of mono- or dizygotic twins The parents of the second pair were available for clinical examination and only the father showed evidence of the disease The haemoglobin genotype in both parents were AS as well as the surviving twin. This information by itself does not confirm or disprove the point that the condition was transmitted as a recessive characteristic However if complete investigations had been possible and it could be shown that the twins though of the same sex were dizygotic this will be strong evidence in favour of an autosomal dominant transmission.

As I indicated there had been some uncertainty about the mode of inheritance in the reported cases and this is why I made the plea for a more detailed family studies investigation and reporting of cases particularly in the African population

Oluwumina Seriki M D
Clinical Affiliate Fellow

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Key words Cutis laxa intrauterine growth retarda-
tion congenital dislocation of the hip

PROCEEDINGS OF PÆDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PÆDIATRIC GASTROENTEROLOGY

Meeting in Lund August 22-24 1970

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been reported. We have investigated whether intestinal damage is present in children with isolated IgA deficiency. Sixteen children over 2 years of age were found to lack serum IgA. Small intestinal biopsy was performed on these children.

Only one of the patients was admitted to hospital because of gastrointestinal symptoms and malabsorption was suspected in another because of severe iron deficiency anaemia. Small intestinal biopsy revealed total villous atrophy in both. The later clinical course was compatible with coeliac disease. In the remaining 14 the morphology of the small intestinal mucosa was normal and in the 8 tested absorptive function was normal. However, all 16 patients had precipitating antibodies to cow's milk.

Direct immunofluorescent studies on both small intestinal and rectal biopsy specimens were performed in 13 patients; in another 2 only one of these specimens was available. No positive fluorescence with anti IgA antiserum was seen in any specimen from the 14 patients. In one case the number of IgA-containing cells was high in both small intestinal and rectal specimens but even in this case IgM-containing cells predominated. This was the only patient whose serum contained trace amounts of IgA (0.5-2.0 mg/100 ml). Faint staining of interepithelial spaces with anti IgM antiserum was evident in the lamina propria of small intestinal specimens and this was more pronounced in the 2 cases with villous atrophy. In rectal specimens clear fluorescence with anti IgM antiserum was seen in the apical portions of the rectal glandular epithelium.

LETTER TO THE EDITOR

ON EXPERIMENTAL TREATMENT OF PREMATURE
ICTERIC INFANTS WITH OROTIC ACID

In the January Issue of this journal (*Acta Paediat Scand* 60 1, 1971) Kintzel, Hinkel and Schwarze report that treatment of premature icteric infants resulted in a decrease in the serum bilirubin level. Orotic acid is a precursor of UDPGA, the co-enzyme of bilirubin conjugation.

Preliminary experiments in our laboratory have shown that orotic acid competes with bilirubin for the binding site on albumin. The displacing effect is stronger than that of salicylic acid. Until the opposite has been proved it would thus be possible to explain the decrease of serum bilirubin concentration as an effect of such displacement. It may be argued that according to the authors the treated infants showed no detrimental effects while displace-

ment of bilirubin from the binding to albumin would most likely precipitate kernicterus in several children. It may still be justified, however, to consider this possible side effect and we fully agree with the authors when they state that it is necessary to examine the question of side effects still more carefully before orotic acid is routinely administered to prevent premature infants from contracting hyperbilirubinaemia.

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The morphological study indicates that the two uncommon conditions, coeliac disease and IgA deficiency, coexist with much higher than random frequency. The presence of precipitating antibodies to cows milk indicates that the integrity of the small intestinal mucosa is in some way disturbed in IgA deficiency.

The immunofluorescent study verified the predominance of IgM containing cells in morphological normal intestinal mucosa demonstrated by others in IgA-deficient coeliac patients. The study also suggests that IgM is secreted onto the intestinal mucosa.

S Auricchio (Naples), F Ciccimarra (Naples), J Jos (Paris), F Rey (Paris), L Moauro (Naples) & J Rey (Paris). *Studies on intestinal digestion of starch in children with congenital deficiency of sucrase and isomaltase activity.*

The analysis of the carbohydrates of intestinal juice after a test meal containing amylopectin in 5 patients, as compared with three normal controls, shows that the salivary and pancreatic alpha amylolysis of amylopectin in the proximal jejunum is normal. After the first year of life, intestinal juice contains glucose, maltose, maltotriose and low and high molecular weight branched dextrans. The intraluminal digestion of the polysaccharide is, on the contrary incomplete both in the patients and in the controls younger than 12 months of age, probably as a consequence of the physiologically low levels of alpha amylase activity. The maltotriose oral tolerance test is normal in a three year old patient, whereas it causes slightly fermentative diarrhoea and a small increase of blood glucose in a 6 month old patient. The mucosal glucamylolytic digestion of starch is impaired in intestinal biopsies of 9 patients probably as a result of the deficiency of glucamylase activity of the sucrase isomaltase complex.

These results suggest that starch malabsorption in congenital sucrase isomaltase deficiency is due to defects in some of the mucosal enzymes involved in the digestion of starch and

of the products of alpha amylolysis of the polysaccharide.

J Schmitz, M Odievre & J Rey (Paris). *Fructose free diet effects on intestinal alpha glucosidase activities in hereditary fructose intolerance.*

Intestinal alpha glucosidase activities of 9 patients affected by hereditary fructose intolerance have been compared with those of 8 normal children. The patients were kept on a fructose free diet for at least 15 months. Biopsy specimens were obtained by the Crosby or the Rubin tubes, enzymatic measurements were performed simultaneously by the Dahlqvist micromethod, on -20°C refrigerated samples.

Normal lactase activity is found in all cases demonstrating good conservation of the mucosa. A significant decrease of about 50% in the sucrase activity and of about 40% in the total maltase activity in the test group is shown as compared with that in the reference group ($p < 0.01$). The activity of maltases 1 and 2 being unchanged the variation of the total maltase activity concerns only the thermolabile fraction.

These results corroborate those previously obtained in rat and man. They confirm that the sucrase activity depends on the quantity of saccharose contributed by the diet. The experimental procedure, however, does not enable us to identify which sugar induces these alpha glucosidase activities.

N G Asp & A Dahlqvist (Lund). *Multiplicity of intestinal beta galactosidases. Contribution of each enzyme to the total lactase activity in normal and lactose intolerant patients.*

Three different beta galactosidases have been demonstrated in the human small intestinal mucosa (1, 2, 3, 4). The first enzyme, the lactase is a brush border enzyme with optimum pH 5.5-6. Lactose is the preferential substrate and 0.2 mM *p*-chloromercuribenzoate (CMB) does not inhibit this enzyme. The second enzyme, the

acid β galactosidase is probably lysosomal and has optimum pH 4-4.5. It has the broadest substrate specificity of the three enzymes hydrolysing a number of synthetic β galactosides as well as lactose and is completely inhibited by 0.1 mM CMB. The third enzyme the hetero- β galactosidase has optimum pH 5.5-6. The enzyme hydrolyses synthetic β galactosides but no lactose and is completely inhibited by 0.1 mM CMB.

The differences between the enzymes regarding pH optimum, substrate specificity and sensitivity to CMB as inhibitor made it possible to work out specific methods that could be used for assay in tissue homogenates without separation of the enzymes. The acid β galactosidase could be estimated with 2 naphthyl β galactoside as substrate since this substrate was not hydrolysed by the other two enzymes. The lactase could be assayed with lactose as substrate in the presence of 0.2 mM CMB as an inhibitor of the acid β galactosidase. The hetero- β galactosidase was assayed with *p* nitrophenyl β galactoside as substrate. The CMB inhibited activity was estimated at pH 6. The contribution of the acid β galactosidase was then calculated from the 2 naphthyl β galactosidase activity and the difference represented the hetero- β galactosidase activity.

In biopsy specimens from 17 lactose intolerant Finlanders (5) the mean activity of brush border lactase was 3.7 U/g protein. The activities of the other two β galactosidases did not differ significantly from those of controls with normal lactase activity. In a group of 18 Zambians (6) the mean activity of brush border lactase was 1.7 U/g protein. The activity of acid β galactosidase was higher than in the lactase deficient Finlanders while the level of hetero- β galactosidase was similar.

Lactase activity is generally assayed at pH 6 where the brush border lactase has maximal activity. The acid β galactosidase will then usually contribute with about 1 unit of lactase activity/g protein. In lactose tolerant patients therefore the acid β galactosidase will be responsible only for 2-10% of the total lactase

In lactose intolerant patients on the other hand up to 90% of the total lactase activity at pH 6 may be due to the acid enzyme. All the lactose intolerant patients studied so far have been adults. Children with congenital lactase deficiency may have even lower or possibly zero levels of brush border lactase.

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K. Launiala, T. Sahl, M. Isokoski, J. Jussila & T. Niemi (Helsinki) *Lactose malabsorption in school-children*

The prevalence of lactose malabsorption (LM) in the adult population (21-65 years old) of the Finnish rural commune of Pornainen was 15% and independent of age (Jussila J, Isokoski M & Launiala K *Scand J Gastroent* 5 49 1970).

The present study concerns the school-children of the same community (7-15 years old). By random sampling 162 children out of 380 were selected. Eighty per cent 130 children consented to participate in the examination. There was no significant difference between the participants and non participants as regards age or sex distribution or regarding abdominal complaints as ascertained by a questionnaire.

An oral lactose tolerance test (1 g per kg body weight with a maximal dose of 50 g) was performed. A rise in blood glucose concentration of at least 25 mg/100 ml was considered a sign of normal lactose absorption. If the rise was 20-24 mg/100 ml the test was repeated with a double dose and a rise of 20 mg/100 ml or more was taken as normal. On every child with a rise of 15-19 mg/100 ml

a small intestinal biopsy was made and mucosal maltase, sucrase and lactase activities were determined. Thus the LM was based on the following criteria: a maximal rise in the lactose tolerance test of less than 15 mg/100 ml, or mucosal lactase activity of less than 15 U/g protein.

LM was found in 11 subjects, the prevalence being 5%, and there was no significant difference in prevalence between boys and girls. In the two age groups, 7-11 and 12-15 years, the prevalence was 6 and 12%, respectively, but the difference was not statistically significant. There was no difference in the occurrence of abdominal complaints, either in general or a day after the examination, between those with LM and those with normal absorption. Nor was there any difference in the height or weight distributions.

When the prevalence of LM, now observed in the two age groups of school children (6 and 12% respectively), is compared with that found in the young adults (23%) of the same community, it seems likely that LM begins to appear even before the age of 7 years but that it most commonly develops between 10 and 20 years.

S Auricchio, M Pierro, G Andria & G De Ritis (Naples) *Arylamidase activities of brush border membrane of rat intestine*

β -Naphthylamide of L-leucine, L-phenylalanine, L-lysine and also α and γ glutamyl β naphthylamide have been used as substrates for the assay of intestinal peptidases. Crude and purified brush border and brush border membrane were prepared from rat intestine.

The pH activity curves are different for the hydrolysis of the different substrates in the membrane preparation: all arylamidase activities are ion dependent, with the exception of that hydrolysing γ glutamyl- β naphthylamide when the activating effect of Mn^{++} , Co^{++} and Zn^{++} was evident only after prolonged exposure of the membrane at 37°C to EDTA. Studies on intracellular distribution demonstrate that

these enzymatic activities of the brush border are due to intrinsic enzyme(s) of the membrane, and these probably play a role in terminal digestion of proteins.

Methods for the assay of these membranous enzymatic activities in total homogenate of intestinal biopsies could be useful in understanding the hypothetical role of intestinal peptidase deficiency in the pathogenesis of diarrhoea.

R Gruttner & Th Lucking (Hamburg) *Chronic diarrhoea following partial resection of the small bowel*

On December 17th, 1967, surgical intervention was necessary on a normally developed girl, born on July 9th, 1965, due to ileus, caused by strangulation of a diverticulum of Meckel. The terminal ileum, the caecum and the appendix, 20 cm in length altogether, had to be resected. Following the operation the girl immediately developed severe diarrhoea and no longer gained in height or weight. Coeliac disease was suggested as the introduction of a gluten free diet in April 1968 was followed by marked improvement in the diarrhoea condition. At this time antibodies against gluten could be demonstrated in the blood. A detailed examination at the end of 1969 in the Children's Hospital of the University of Hamburg did not point to any of the chronic intestinal malabsorption syndromes known at the present time. All resorption tests performed were normal. Steatorrhea could not be demonstrated. Oral loading with gluten was followed by diarrhoea, apart from intolerance to gluten no other cause for this could be found. Diarrhoea continued for several weeks during which steatorrhea could once more not be demonstrated. Oral biopsies following the oral gluten loading did not show pathognomonic changes of the intestinal mucosa resembling those of gluten intolerance. Two months later oral loading with milk protein was performed. Abdominal pain and partly mushy stools occurred but again steatorrhea could not be seen. The renewed oral biopsy showed partial and subtotal

villous atrophy There were regions with almost total loss of villi close to regions with very small villi the tops of which were covered with irregular epithelial cells The clubbed thickenings of these villi showed lymphangiectatic vessels Following a gluten and milk free diet marked improvement of diarrhoea occurred

Discussing the mechanism of the pathogenesis an inborn or constitutional disease can be excluded for there was no sign of food intolerance prior to surgery This case reminds us of severe intestinal infections in the neonatal and in early infancy In these infections exogenous mechanisms may also cause coeliac disease like syndromes This may be due to unspecific food intolerance It is impossible to determine whether the pathogenesis of this disease is influenced by bacterial migration from the colon to the small intestine or by disturbances of the reabsorption of bile salts

J Jodl Z Lojda N Šumančková & P Fric (Prague) *Involvement of the small intestine in cystic fibrosis*

Jejunal biopsies of 18 children with cystic fibrosis were investigated histologically histochemically and biochemically Histologically the mucosal pattern can be divided into 4 groups In the 1st group (9 patients) no histological histochemical and biochemical abnormalities were found In the second group (3 patients) the mucosal pattern was slightly irregular with some deeper crypts and lower villi—the enterocytes displayed an enhanced quantity of RNA but their microvillous zone was morphologically and cytochemically normal In the 3rd group (3 patients) the mucosal pattern was also normal but the number of goblet cells was increased The crypts were enlarged and hypersecretion of a viscous mucus which adhered also to the villi was observed In the 4th group (3 cases) a typical pattern of coeliac sprue was found Total or subtotal atrophy of the villi deeper crypts defective microvillous zone of enterocytes with lower enzyme activities (disaccharidases alkali

line phosphatase, aminopeptidase), enhanced quantity of cytoplasmic RNA lower activities of non specific esterase glucose 6-phosphatase and lower activities of lysosomal enzymes In the propria cellular infiltration consisting of activated macrophages plasmocytes lymphocytes and eosinophils was found Typical changes were found also in zymograms shift of LDH isoenzymes in form of "H subunits and a deficiency of cathodic fractions of AF and aminopeptidase An improvement after a gluten free diet was observed

It is concluded that malabsorption in cystic fibrosis need to be only of pancreatogenic origin Altered intestinal mucosa may participate as well In some cases even a concomitant coeliac sprue can occur and this must be taken into account in the treatment of the patients

Helga Stolley & W Droese (Dortmund) *Lactic acid in milk formula the influence on absorption of nutrients and the influence on the metabolism in young babies*

In the course of long term balance studies with healthy babies during the first 3 months of life we added 0.35 to 0.5 g lactic acid to 100 g milk formula for ten-day periods Two different formulae were given The protein poor formula consisted of one or two parts of cow's milk diluted with one part of water The protein rich formula consisted of four parts of milk and one part of water

After addition of lactic acid to the milk formula excretion of nitrogen lipids potassium sodium and calcium increased significantly in the stools The acidity of urine increased to pH 4.5 Organic acids were excreted to higher extent Ammonia and titrable acidity were increased during the lactic acid period by 100% and more Calcium and magnesium urinary excretion were increased 30% of the infants under study developed a metabolic acidosis including distinct clinical signs (paleness vomiting and retarded weight gain)

According to our findings the influence of lactic acid on the utilization and on the metabolism is greater the higher the protein con

a small intestinal biopsy was made and mucosal maltase, sucrase and lactase activities were determined. Thus the LM was based on the following criteria: a maximal rise in the lactose tolerance test of less than 15 mg/100 ml, or mucosal lactase activity of less than 15 U/g protein.

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II Hadorn (Bern) Ch. Sumida M J Tarlow L Robinson & T T White (Seattle) *The activation of human pancreatic proteinases. Studies on human pancreatic juice and duodenal juice of four children with intestinal enterokinase deficiency*

The activation of pancreatic proteinases in man has been studied using purified human enterokinase for trypsinogen activation and human pancreatic juice as the source of zymogens. The same process has been studied in duodenal juice from patients with enterokinase deficiency as it contains pancreatic zymogens instead of active proteinases.

In human pancreatic juice two molecular species of trypsinogen are present as has been shown previously by Figarella (1) and Keller (2). The two forms of trypsin found in human duodenal juice (3) appear to be derived from these two zymogens rather than to be activation products of the same zymogen. The two trypsinogens have isoelectric points of approximately 4.7 and 7.0 as judged from their behaviour in the electrofocusing apparatus. Both trypsinogens are activated by enterokinase. The two trypsinogens appear to occur with different frequencies in the population.

Duodenal juice of patients with intestinal enterokinase deficiency offers the unique possibility of studying the activation process under "physiological" conditions. Activation studies were made with duodenal juice from 4 such patients. It is confirmed that enterokinase deficiency is the only cause of non activation of pancreatic zymogens. Autoactivation of trypsinogen in the patients' juice is prevented by a trypsin inhibitor which is probably identical with the trypsin inhibitor described by Keller in normal pancreatic juice.

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Ch. Nordstrom & A Dahlqvist (Lund) *The cellular and subcellular localization of enterokinase*

Enterokinase (enteropeptidase, E.C. 3.4.4.8) which activates pancreatic trypsinogen in the small intestine is usually assumed to be secreted from the intestinal mucosa with the intestinal juice. When we performed a study of the localization of enterokinase in the intestinal wall in order to define the site of its origin we found a localization which clearly indicates that enterokinase is not secreted from the deep layers of the mucosa but is located on the surface of the mucosal absorptive cells.

The study was performed in rats. When a piece of rat small intestine was cut out and rinsed about 90% of the total trypsin activity but less than 5% of the enterokinase activity was removed. When pieces of the duodenal wall were cut horizontally in a cryostat (1, 2, 3, 4) in order to obtain sections from different levels in the villi and crypts respectively, the major part of the enterokinase activity was found in the tips of the villi. None of a series of pieces studied from different parts of the duodenum revealed any demonstrable enterokinase activity in the crypts or in the Brunner glands. The distribution profile along the villi for alkaline phosphatase activity was as apical as that of enterokinase activity while the sucrase activity was more basally located in the villi and decreased in the tips.

We also isolated brush borders from the duodenal epithelial cells using essentially the method described by Miller & Crane (5). The specific enterokinase activity (units per mg of protein) was more than 10 times higher in the isolated brush border fractions than in the original homogenates.

In spite of the fact that enterokinase thus like the disaccharidases is a brush border enzyme proportionally much greater amounts of enterokinase than disaccharidases were found in human duodenal juice after a test meal indicating that the functional and anatomical localization of enterokinase may not be identical. If the enterokinase activity is presented

tent of the milk formula and the younger the infant

The prevalence of metabolic acidosis with the high protein formula could be explained by the fact that increased protein intake is equivalent to increased intake of organic acids and consequently increased metabolic rate of organic acids

A second programme was started in order to discover the reasons for the poor tolerance to lactic acid in young babies. The lactic acid used constitutes 80% L (+) and 20% D (-) lactic acid. We examined the excretion of a number of organic acids in urine under non-acidified and under acidified cow's milk mixtures. From day 6 after birth till the end of the third month lactic acid constitutes less than 1% of the total organic acids. After addition of 0.35 g of lactic acid per 100 g milk formula there was an increased excretion of organic acids in urine. Lactic acids increased from less than 1% to 10% of the total organic acids. There was a decrease of citric acid during the lactic acid feeding period. The excretion of lactic acid in urine is mainly D (-) lactic acid (80%).

During our study we observed one infant with severe lactic-acid intolerance.

The results led to the following conclusions:

- 1 lactic acid is metabolized more slowly the younger the infant
- 2 D lactic acid is utilized by the infant to a much lower degree than L lactic acid
- 3 D lactic acid is supposedly responsible for the detrimental effects of the addition of lactic acid to cow's milk mixtures
- 4 Excretion of citric acid in urine was found to be a sensitive indicator for the acid base metabolism of the infant

U Spahn, Ursula Eltz & W. Plenert (Jena)
Serum lipid pattern during infectious hepatitis in children. Influence of dietary management

The acute phase of infectious hepatitis was associated with remarkable changes in serum lipids even in patients with less severe course

of this disease. Since nearly all lipid classes were affected in a different manner, the observed biochemical abnormalities are difficult to interpret.

The serum free fatty acids (FFA) were found to be significantly increased initially in most of the cases. In agreement with the clinical aspects and serum transaminases they returned to normal values in the second and third week of the treatment, respectively. No differences could be established concerning this parameter between children treated with a "usual liver diet" and those, who were on a "normal diet" containing butter fat even in the beginning of treatment. Esterified cholesterol of all fractions showed a significant fall, while free cholesterol was found to be markedly increased.

Thus the ratio cholesterol ester/total cholesterol was considerably diminished to an average of 30 compared to 65 in normal children. Within 3 weeks of hospitalization this ratio returned to normal, while the relative distribution of cholesterol esters remained abnormal, since there was now a decreased percentage of linoleate in all children, who had been on a "liver diet". In only 5 of the patients fed with a normal diet had the percentage of cholesterol esters become normal. The initial drop in esterified cholesterol may be due to a reduced rate of cholesterol esterification while the decrease in cholesterol linoleate may be due to an insufficient fat supply. In contrast to these changes in cholesterol ester concentration total esterified fatty acids in serum tended to be elevated. They seemed however to be largely unaffected by dietary management. Serum phospholipids were affected in a characteristic manner for acute hepatitis.

There were reasonably close correlations between total cholesterol, free cholesterol, total lipid phosphorus and lecithine. Sphingomyelin and lysolecithin rose somewhat and lecithin rose markedly. The abnormalities in serum phospholipids are suggested to be mainly due to the metabolic disorders in the diseased liver. To some extent they may be caused by a transitory bile retention.

probably represent the maximal evacuation power of the infant stomach at this age

It is concluded that within a few minutes after feeding the young infant glucose or milk meals there are mechanisms operating which slow down the gastric emptying rate so that the stomach empties 2 to 3 times below its maximal capacity. As the influence of the volume varies for different kinds of meal gastric emptying rate seems to be determined by the interaction between volume dependent gastric propulsive activity and inhibitory mechanisms influenced by the composition of the meal

F Drillet F Rey & J Rey (Paris) *Influence of the rate of perfusion on the in vivo kinetics of glucose and amino acid absorption*

A technique of intestinal perfusion with a double lumen tube was applied to 24 children ranging from 6 months to 5 years age. Glucose leucine and lysine solutions (rendered isotonic by addition of NaCl) were infused in the first jejunal loop. Intestinal absorption was calculated on 20 cm

Analysis of the influence of the rate of infusion (1.2-2.8-3.6 and 4.5 ml/min) has demonstrated that intestinal absorption increases in proportion to the flow and that this is not related to any modification in the concentration of the substrate along the segment examined. A study on the kinetics of absorption at two different rates (3.8 and 7.5 ml/min) has shown moreover that absorption is not proportional to the substrate concentration but depends on the number of molecules perfused per unit of time: the maximum absorption capacity is identical for the two rates eliminating the hypothesis that this effect may be related to a variation in the surface of the studied loop.

The mechanism of intestinal absorption of glucose and amino acids in an open system is therefore identical to that of the kidney (Tm limited). The concept of Km has in this case no significance the system being defined by its maximum capacity and its half saturation loading.

H Loeb G Vandeveld S Godart M Goldstein A Piepsz & J Otten (Brussels) *Protein losing enteropathy with anomalies of intestinal lymphatics: diagnosis and treatment*

The case report concerns a girl aged 12 years presenting progressive generalized oedema and vague intermittent abdominal pain. Laboratory data revealed a hypoproteinemia without albuminuria as well as a lymphocytopenia. Nutritional deficiency, kidney and liver diseases were ruled out. Intestinal protein loss was demonstrated with ¹³¹I labeled albumin and albumin ⁵¹Cr. Intestinal transit and jejunal biopsy were normal. A lymphography disclosed generalized lymphangiectasia with reflux in dilated mesenteric and coeliac vessels. The diagnosis was confirmed at laparotomy and the dilated prepancreatic vessels were ligated and cut. Two months afterwards serum proteins became normal and isotopic tests confirmed the disappearance of the intestinal protein loss. However 5 months after the operation a chylous ascitus developed progressively; serum proteins remained normal. A peritoneo-venous anastomosis was carried out which proved unsuccessful after 2 weeks. Thereafter ganglio-venous anastomoses were undertaken allowing a satisfactory lymphatic drainage; ascitus disappeared gradually.

L L Kulczycki (Washington) *Malabsorption with vitamin A deficiency in a college girl treated for cystic fibrosis*

White female 19 years old wt. 46 kg ht. 155 cm (5-10) a known case of cystic fibrosis with excellent progress until recently was hospitalized with acute bronchial pneumonia.

Following admission these additional manifestations were revealed: (a) night blindness and diminished day vision deteriorating significantly within the past 3 months; (b) anosmia; (c) amenorrhoea for the past 8 months; (d) absent DTR and questionable ataxic gait; (e) subclinical malabsorption. A suggestion of brain

to the intestinal lumen by cell desquamation or by some hitherto unknown release mechanism acting on intact cells in the villi remains to be explained

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G Zoppi, G Andreotti, F Pajno-Ferrara & D Gaburro (Ferrara) *Pancrozymin-secretin test in newborns*

Pancreatic response to pancrozymin and secretin stimulations has been studied in 24 newborns 16 were premature with a gestational age between 32 and 34 weeks and did not present any evidence for respiratory or neurological illness Their weight at birth was between 2 and 2.4 kg 8 were full term healthy babies with a weight at birth between 3.6 and 4 kg

Duodenal contents were collected through a newly manufactured double balloon triple lumen plastic PVC tubing having an external diameter of 3.5 mm

The patients all receiving the same diet were tested three times, i.e. at birth before first feeding during second day of life and at end of first week. In premature babies an additional test was performed at 1 month of age

Total output of fluid volume and pH were measured immediately after completion of the test, and trypsin lipase and α -amylase activities were then assayed and calculated as output/kg body weight/50 min after hormonal stimulations

The measured enzyme activities present at birth in both premature and full term babies seem to diminish during first day of life and return to starting values at one week

At birth the enzyme activities found in premature babies are lower than in full term babies, and become higher 1 week later. The possible role of various adaptation mechanisms is discussed in order to explain the observed patterns of pancreatic response to pancrozymin and secretin stimulations

B Cavell (Lund) *Gastric emptying in infants*

Gastric emptying patterns were studied in infants aged 2 to 12 weeks after the administration of a meal of human milk. After giving the milk via an infant feeding tube, reiterated intragastric volume determinations were performed at regular intervals using a marker (polyethylene glycol). Most of the infants showed an initial, rapid emptying phase where the stomach emptied 1 to 3 ml of its contents per minute. This was followed by a slower phase with a more or less constant emptying rate about 0.3 to 0.7 ml/min until the stomach was empty. The basic emptying pattern could best be described by an exponential function with 1 to 3% of the gastric contents emptying per minute.

The effect of varying the volume and the composition of the test meal on the emptying rate during the first ten minutes was examined in 6 infants. Each infant was given three different volumes of 200 milliosmolar saline, 5% glucose and 10% glucose, respectively, so that the influence of the quantity and the quality of the meal could be evaluated simultaneously in the same infant. The emptying rate proved to be dependent of the volume of the meal, i.e. the larger the volume the faster the emptying of the stomach. This was most marked for the saline meals where a 10 ml increase in the volume raised the emptying rate by an average of 0.63 ml/min. The corresponding figures for 5% and 10% glucose were 0.32 and 0.30, respectively, and, when calculated for the milk meals as a comparison, 0.16 ml/min. For large meals of saline, emptying rates of about 10 to 12 ml/min were recorded. Such high rates

to severe malnutrition after being fed cows milk. In three of these children absorption studies were performed. Steatorrhea was present whereas lactic acid excretion was normal. Jejunal biopsies were in all 4 cases completely or almost completely flat.

In one case withdrawal of cows milk and its replacement by soyabean milk was followed by clinical improvement. At 5 months a repeat jejunal biopsy showed normal villous morphology.

The second case was given human milk, medium chain triglycerides, fructose and pancreatic enzymes. The clinical picture improved very slowly. There was no immunological defect. At 12 months weight corresponded to the tenth percentile for age but height was below this standard. At this age the villous morphology was almost normal.

The third case had received several antiseptic drugs before admission. Sepsis due to colibacillus and intestinal infection due to O55B5 Colibacillus were demonstrated. Diarrhoea could not be controlled by discontinuing oral feeding. The patient died after surgical intervention of acute intestinal obstruction.

In the fourth case an intestinal infection by Colibacillus was noted but diarrhoea preceded infection and persisted after its cure. Withdrawal of cows milk and its replacement by soyabean milk, medium chain triglycerides and fructose coincided with clinical improvement and moderate weight gain.

S Nordio, I Antener, M Kaser & R. Gatti (Genoa) *Aminoacids of faecal ultrafiltrate in different pathological conditions*

Ultrafiltrates of 24-hours faeces from a few control subjects and some cases with different diseases (common rickets, vitamin D resistant rickets, pseudodeficiency vit D-resistant rickets, Hartnup syndrome, cystinuria, Lowe syndrome, maple syrup disease etc) were studied with the Stein Moor method.

N Ansaldi & A. Musso (Torino) *Gastric juice immunoglobulin values in the first two years of life*

Gastric juice immunoglobulin evaluation in healthy children in the first two years of life showed a prevalence of IgM.

This finding conflicts both with the literature data reported for duodenal and intestinal secretions in the adult and with immunological pictures observed in older controls in the present study.

H J Schmidt, J Wehba, N Carvalho, J Pinus & A Carvalho (Sao Paulo) *Radioactive (RBI¹³¹) rose Bengal excretion test in the obstructive jaundices of the infants*

Urine and stools were collected strictly separated for a period of 72 hours divided in 3 periods of 24 hours collection. Six infants were used for control being 5 neonates 3 to 4 days old and one 65 days old. The patient group constituted 46 patients: 11 cases of biliary atresia, 27 cases of temporary obstructive jaundice, 1 case of inspissated bile syndrome, 1 case of biliary atresia with situs inversus totalis and supra-numerary spleens.

The very early age and jaundice do not represent any contraindication for the use of the RBI¹³¹ excretion test. Urinary excretion was of no importance. The results obtained indicate the accuracy of the faecal RBI¹³¹ uptake determinations as a reliable test in the differential diagnosis of obstructive jaundice of infants.

CROHN'S DISEASE AND ITS RELATION TO ULCERATIVE COLITIS

■ O Berg (Lund) *Morphologic aspects*

E Boysen (Lund) *Angiographic characteristics*

R. Lagercrantz (Stockholm) *Immunological aspects*

Most patients with ulcerative colitis have elevated humoral antibody titres to antigen(s) from

tumor was ruled out by prompt neurological consult

Ophthalmological consult confirmed visual acuity right 20/80 and left 20/50, xerosis of cornea and conjunctiva bilaterally, and elevated dark adaptation with reduced rod and cone function to 30% measured by electroretinogram (ERG). The other laboratory data revealed *pneumonic infiltrations* by X-ray. Liver function tests—borderline, with total bilirubin 2, indirect 1.75. Absorption studies, D-xylose, 5 hours urine excretion 15% (borderline). Vitamin A fasting level 19 $\mu\text{g}/100\text{ ml}$, after loading with Vitamin A 66 $\mu\text{g}/100\text{ ml}$ (normal 150 $\mu\text{g}/100\text{ ml}$). Loss of 33% of fat intake and loss of 22% of protein. Serum cholesterol level 166 $\text{mg}/100\text{ ml}$ and plasma triglyceride 120 $\text{mg}/100\text{ ml}$, both normal.

In addition to her usual diet, with pancreatic enzymes and multivitamin supplements, the patient was put on 100 000 units oral Vitamin A daily, and after 1 month showed marked clinical and symptomatic improvement. Repeat ophthalmologic examination revealed visual acuity right eye 20/30, left 20/30, conjunctiva moist with good lustre. There was no longer any epithelial oedema or corneal staining. Blind spot was still enlarged. Repeat ERG showed rod function still only 30% of normal, but cone function was the lower limit of normal. Schirmer test revealed test strip moistened in three minutes.

This patient exhibited all features of Vitamin A deficiency and cystic fibrosis.

II Vitek O Saxl & O Teyschl Jr (Brno)
Investigation of liver metabolism in mongolism by means of catheterization

To study the metabolism of carbohydrates and lipids in Down's Syndrome we catheterized mongoloid children aged 2–12 years (4 trisomies, 2 translocations and 1 mosaicism). 6 were given glucagon and 1 epinephrine. Blood samples from the V cava sup and from the V hep were analysed for glucose, lactate, pyruvate and non esterified fatty acids levels.

After stimulation, hyperglycaemia was found in the V hep and in the V cava sup, the former being more marked. The highest elevation occurred after 15 min, the peak level being reached between 30–60 min. After 90 min in 2 subjects only (1 translocation and 1 mosaicism) the glucose level had returned to normal in both blood samples. In the other subjects the glycaemia was still highly increased.

Thus the glucogenolysis after glucagon or epinephrine did not differ from the normal response, indicating that the glycogen content of the liver is normal in spite of the fatty infiltration.

While investigating the levels of lactate and pyruvate 5 subjects showed after 12 hours fasting elevated levels of lactate in both samples, the blood levels in the V hep being three the normal amount. In all children glucagon or epinephrine lowered lactate in the V hep to subnormal levels within a few minutes, the systemic levels being only slightly affected.

The pyruvate levels were investigated in 4 children. Glucagon or epinephrine caused a decrease during the first 15 min. The amount of non esterified fatty acids was also elevated in both blood samples, more in the V cava sup. After glucagon or epinephrine the reaction of the patients was the same as that of healthy children.

The results confirm the existence of an abnormal regulation of carbohydrate metabolism but we did not find any changes in lipolytic response in our patients. For the time being we cannot explain the occurrence of the high fasting hyperlactaemia. As it prevails in the hepatic vein, insufficient utilization and metabolism in the liver is probable.

H Loeb M Vansel S Cadranet, A Dachy, M Diedrickx & R Wolter (Brussels)
Malabsorption and villous atrophy in four infants relationship to cow's milk intolerance

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may be an extremely sensitive indicator of terminal ileal disease and the ratio is considered to be of great value as a diagnostic and consequently a prognostic guide in patients with inflammatory bowel disease

E Hess Thaysen (Aalborg) *Long term prognosis in Crohn's disease*

The epidemiology and course of Crohn's disease (Cd.) is difficult to determine since the diagnostic criteria and statistical methods used vary from series to series

The disease may manifest itself at any age and differences in sex incidence are small. Clinical onset before the age of 10 is rare (2-3%). In approximately 80% of the cases Cd develops in the second to fourth decade of life. During early youth the disease tends to run a more fulminant course.

In about 50% of the cases the inflammatory process is limited to the small intestine, in 10% to the colon and in 40% it involves both organs.

Cd is a capricious disease that is usually slowly progressive. Many patients may however experience long periods of clinical remission. In most cases complications such as stenosis, the development of fistulae or abscess formation will eventually necessitate surgery. The frequency of recurrence after apparently curative major surgery increases with the period of observation and is usually quoted to be in the range of 50%. Those patients requiring a second or third resection have a 50% chance of reasonable recovery after each operation.

About 25 cases of malignant tumours in the small intestine have been described in association with Cd. This is not sufficient for statistical evaluation but nevertheless it should lead to some reflections on a possible connection between the two diseases. In Cd of the colon the incidence of carcinoma is 0.5% as opposed to 9.0% in prolonged ulcerative colitis (overall incidence is 0.06%). Thus in both diseases the incidence of malignancy is significantly increased.

In several series usually covering a span of three to four decades, the mortality in Cd is estimated to be about 10%. A specific mortality rate cannot be assessed from these surveys. In a recent paper (*Lancet* I 1135 1970) the sex, age and years at risk for each patient has been considered in order to evaluate the death rate. The mortality registered was more than twice the number expected for a normal population of similar age and sex distribution. The excess number of deaths was significant for both sexes and for those who had developed symptoms before the age of forty.

G W Meeuwisse & B Hansing (Lund) *Medical versus operative treatment*

A retrospective study has been carried out on the cases of regional enteritis (Crohn's disease) (RE) and ulcerative colitis (UC) admitted for the first time before the age of 15 to the Lund hospital during two consecutive 7 year periods: 1956-62 and 1963-69. It was attempted to make exact diagnoses on the available data. In the first period there were 2 sure cases of RE (2 girls), 1 doubtful case, 2 cases of either RE or UC and 2 sure cases of UC (2 boys). In the last 7 year period the incidence of both diseases was higher: 14 cases of RE (10 boys, 4 girls) and 17 cases of UC (3 boys, 14 girls). In this period the sex distribution corresponds to most reports in the literature. During the first 7 year period RE was not treated with long term administration of drugs. The RE patients were treated surgically. Several relapses requiring new resections occurred and as malnutrition developed the results were disappointing.

Since 1963 RE patients have been treated with Salazopyrin except in one case, a girl who due to acute initial illness was taken over to the surgical department, resected and considered cured. The other patients have hitherto been well or much improved. Some of them have been given small doses of steroids as well as Salazopyrine during limited periods. In some illustrative cases discontinuation of the Salazo-

colon. These autoantibodies crossreact with antigen(s) from *Escherichia coli*. Patients' leukocytes are cytotoxic in vitro to colon mucosa cells. Enteric antigen(s) inhibit in vitro migration of patients' leukocytes.

Antibodies against colon are also elevated in most patients with Crohn's disease. These antibodies probably have different specificity than those found in patients with ulcerative colitis. In vitro migration inhibition tests are reported negative in Crohn's disease. According to some investigators, patients with this disease also have impaired delayed hypersensitivity to tuberculin and Kveim's antigen.

Both diseases respond fairly well to Salazopyrine, corticosteroids and immunosuppressives but probably require radical surgery for definite cure.

The very marked difference in the incidence of malignancy in these two diseases may reflect different immunological mechanisms.

E. Hess-Thaysen (Aalborg) *Some reflections on the diagnosis of Crohn's disease*

Diagnostic uncertainty is in particular related to the problem of differentiating Crohn's disease of the colon from ulcerative colitis. None of the features frequently referred to as more or less characteristic are in themselves pathognomonic and, in spite of firm clinical criteria, correspondance with pathological features is rather poor. Consequently it has been proposed that in the clinical treatment of such patients a determination of the site and extent of the disease should be considered more valuable as a prognostic guide than the clinical label attached to it. In this context the demonstration of possible terminal ileal disease is of particular interest.

Small intestinal radiography and selective angiography are often most helpful but these examinations may occasionally fail to disclose definite diagnostic features in this part of the gut. In such cases more precise information may be derived from a detailed evaluation of the different functions of the terminal ileum.

As is well known a pathological Schilling test and perhaps a low concentration of serum cholesterol may be compatible with a terminal ileopathy. This may also apply to a normal D-xylose test in the presence of steatorrhoea. During recent years the interest has been further focussed on the disturbed enterohepatic circulation of bile salts in disease of the terminal ileum. Recently, evidence of this type of disturbance has been found in the form of a raised glycine/taurine conjugation ratio of bile salts in aspirates from the duodenum.

A. Bruusgaard (Aalborg) *Localization of inflammatory bowel disease as a prognostic guide*

The evaluation of the prognosis in Crohn's disease is not an easy matter, chiefly due to the diagnostic difficulties, especially concerning the differentiation of Crohn's disease of the colon from ulcerative colitis.

According to Giotzer et al. (*New Engl J Med* 282:582, 1970) a determination of the site and extent of the inflammatory bowel disease seems to be the most valuable prognostic guide.

With regard to the site, the main difficulty, but at the same time the most important factor, is usually to demonstrate the presence of terminal ileal involvement, since the conventional methods may fail.

In terminal ileopathy the enterohepatic circulation of bile acids is disturbed, resulting in an increased ratio of glycine to taurine conjugated bile acids (G/T ratio) in the bile.

A report is given of two patients with diarrhoea and slight steatorrhoea due to inflammatory bowel disease localized in the terminal ileum. Their G/T ratio was demonstrated to be increased even before the conventional methods showed evidence of terminal ileopathy. The diagnosis of Crohn's disease was later confirmed by operative and microscopic findings.

Bile acid analysis was performed according to the procedure described by Bruusgaard (*Clin Chim Acta* 28:495, 1970).

It is concluded that an increased G/T ratio

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Diagnostic uncertainty is in particular related to the problem of differentiating Crohn's disease of the colon from ulcerative colitis None of the features frequently referred to as more or less characteristic are in themselves pathognomonic and, in spite of firm clinical criteria correspondance with pathological features is rather poor Consequently it has been proposed that in the clinical treatment of such patients a determination of the site and extent of the disease should be considered more valuable as a prognostic guide than the clinical label attached to it In this context the demonstration of possible terminal ileal disease is of particular interest

Small intestinal radiography and selective angiography are often most helpful, but these examinations may occasionally fail to disclose definite diagnostic features in this part of the gut In such cases more precise information may be derived from a detailed evaluation of the different functions of the terminal ileum

As is well known a pathological Schilling test and perhaps a low concentration of serum cholesterol may be compatible with a terminal ileopathy This may also apply to a normal D-xylose test in the presence of steatorrhoea During recent years the interest has been further focussed on the disturbed enterohepatic circulation of bile salts in disease of the terminal ileum Recently, evidence of this type of disturbance has been found in the form of a raised glycine/taurine conjugation ratio of bile salts in aspirates from the duodenum

A Bruusgaard (Aalborg) *Localization of inflammatory bowel disease as a prognostic guide*

The evaluation of the prognosis in Crohn's disease is not an easy matter, chiefly due to the diagnostic difficulties especially concerning the differentiation of Crohn's disease of the colon from ulcerative colitis

According to Glotzer et al (*New Engl J Med* 282 582, 1970) a determination of the site and extent of the inflammatory bowel disease seems to be the most valuable prognostic guide

With regard to the site the main difficulty, but at the same time the most important factor, is usually to demonstrate the presence of terminal ileal involvement since the conventional methods may fail

In terminal ileopathy the enterohepatic circulation of bile acids is disturbed, resulting in an increased ratio of glycine to taurine conjugated bile acids (G/T ratio) in the bile

A report is given of two patients with diarrhoea and slight steatorrhoea due to inflammatory bowel disease localized in the terminal ileum Their G/T ratio was demonstrated to be increased even before the conventional methods showed evidence of terminal ileopathy The diagnosis of Crohn's disease was later confirmed by operative and microscopic findings

Bile acid analysis was performed according to the procedure described by Bruusgaard (*Clin Chim Acta* 28 495, 1970)

It is concluded that an increased G/T ratio

may be an extremely sensitive indicator of terminal ileal disease and the ratio is considered to be of great value as a diagnostic and consequently a prognostic guide in patients with inflammatory bowel disease

E. Hess Thaysen (Aalborg) *Long term prognosis in Crohn's disease*

The epidemiology and course of Crohn's disease (Cd) is difficult to determine since the diagnostic criteria and statistical methods used vary from series to series

The disease may manifest itself at any age and differences in sex incidence are small. Clinical onset before the age of 10 is rare (2-3%). In approximately 80% of the cases Cd develops in the second to fourth decade of life. During early youth the disease tends to run a more fulminant course.

In about 50% of the cases the inflammatory process is limited to the small intestine, in 10% to the colon and in 40% it involves both organs.

Cd is a capricious disease that is usually slowly progressive. Many patients may however experience long periods of clinical remission. In most cases complications such as stenosis, the development of fistulae or abscess formation will eventually necessitate surgery. The frequency of recurrence after apparently curative major surgery increases with the period of observation and is usually quoted to be in the range of 50%. Those patients requiring a second or third resection have a 50% chance of reasonable recovery after each operation.

About 25 cases of malignant tumours in the small intestine have been described in association with Cd. This is not sufficient for statistical evaluation but nevertheless it should lead to some reflections on a possible connection between the two diseases. In Cd of the colon the incidence of carcinoma is 0.5% as opposed to 9.0% in prolonged ulcerative colitis (overall incidence is 0.06%). Thus in both diseases the incidence of malignancy is significantly increased.

In several series usually covering a span of three to four decades the mortality in Cd is estimated to be about 10%. A specific mortality rate cannot be assessed from these surveys. In a recent paper (*Lancet* 1135 1970) the sex, age and years at risk for each patient has been considered in order to evaluate the death rate. The mortality registered was more than twice the number expected for a normal population of similar age and sex distribution. The excess number of deaths was significant for both sexes and for those who had developed symptoms before the age of forty.

G. W. Meeuwisse & B. Hansing (Lund) *Medical versus operative treatment*

A retrospective study has been carried out on the cases of regional enteritis (Crohn's disease) (RE) and ulcerative colitis (UC) admitted for the first time before the age of 15 to the Lund hospital during two consecutive 7 year periods: 1956-62 and 1963-69. It was attempted to make exact diagnoses on the available data. In the first period there were 2 sure cases of RE (2 girls), 1 doubtful case, 2 cases of either RE or UC and 2 sure cases of UC (2 boys). In the last 7 year period the incidence of both diseases was higher: 14 cases of RE (10 boys, 4 girls) and 17 cases of UC (3 boys, 14 girls). In this period the sex distribution corresponds to most reports in the literature. During the first 7 year period RE was not treated with long term administration of drugs. The RE patients were treated surgically. Several relapses requiring new resections occurred and as malnutrition developed the results were disappointing.

Since 1963 RE patients have been treated with Salazopyrin except in one case, a girl who due to acute initial illness was taken over to the surgical department, resected and considered cured. The other patients have hitherto been well or much improved. Some of them have been given small doses of steroids as well as Salazopyrin during limited periods. In some illustrative cases discontinuation of the Salazo-

pyrine treatment was followed by recurring symptoms and weight loss. Reinstated therapy resulted in remission. In one case, where a drug induced exanthema developed, discontinuation has not given new symptoms although X ray visible changes persist. The other cases remain on treatment except one, a girl, where Salazopyrine was discontinued by the surgeon when, due to stenosis, her left colon was resected. She has not been well since operation. The other cases have not yet needed surgical treatment.

Considering the high recurrence rate of RE after resection and the fact that microscopic changes are detectable in the gut mucosa far away from the macroscopic lesion in many of the cases, the disease ought to be regarded as a chronic, systemic disease. Long term medical treatment will be most beneficial when started early in the course of the disease. A localized lesion may be resected, especially if obstruction is threatening, but it seems worthwhile to continue Salazopyrine treatment in such patients as well.

J. T. Harries & June K. Lloyd (London)
Azathioprine in the treatment of Crohn's disease

We present 2 children with severe and extensive intestinal involvement who improved markedly after treatment with azathioprine in combination with a small dose of prednisolone.

Case 1 The diagnosis was made at 10 years when she presented with a short history of bloody diarrhoea, weight loss and abdominal pain. Initially she responded to salazopyrine and steroids but these drugs had little effect when she subsequently relapsed a year later. Symptoms persisted, her nutritional state deteriorated, severe perianal fissures developed and signs of steroid toxicity developed. Investigations at 12 years showed the distal ileum transverse and descending colon to be involved. Haemoglobin was 8.5 g/100 ml and serum iron, folate and total protein levels were reduced, faecal fat was 9.6 g/day and ESR 27 mm in

1 hr. Azathioprine (2 mg/kg/day) and prednisone (0.2 mg/kg on alternate days) resulted in a striking clinical and biochemical improvement after 8 weeks. The dose of both drugs were gradually reduced without clinical deterioration, but when the prednisone was stopped after 13 months there was a prompt relapse.

Case 2 A 4-year old boy whose father also had Crohn's disease presented with a short history of weight loss, diarrhoea and abdominal pain. There was extensive involvement of the small intestine, haemoglobin, serum iron, folate and albumin levels were reduced, faecal fat was 5.3 g/day and ESR 52 mm in 1 hr. The response to azathioprine (2 mg/kg/day) and prednisolone (0.5 mg/kg/day) was striking. When the prednisolone was reduced to 0.1 mg/kg/day symptoms promptly recurred and rapidly disappeared when the dose was increased to 0.3 mg/kg/day. After 14 months of combined therapy his condition remains good.

K. H. Schafer & F. Blaker (Hamburg)
Azathioprine in the treatment of ulcerative colitis

In the conservative long term therapy of ulcerative colitis: psychotherapy and Salazopyrine still hold an important place. Immunosuppression is on the other hand a new and obviously very effective therapeutic means. As the first remedy we have chosen Azathioprine. However, Azathioprine needs some weeks until it reaches its full effectiveness. Therefore at the beginning of therapy we combine the Azathioprine dose (5 mg/kg/day) with 6 methyl prednisolone (1 mg/kg/day) which is quickly effective. After onset of remission (usually within four weeks) 6 methyl prednisolone is slowly removed and Azathioprine is reduced to the maintenance dose of 3 mg/kg/day. Thus therapy has been used in our hospital since 1966 for ulcerative colitis in children and we have obtained thoroughly satisfactory results. Out of 14 cases there are at the moment 12 in satisfactory remission of 2 till 32 months. A 13th case unaccountably relapsed under maintenance therapy of 3 mg/kg/day Azathioprine after a

satisfactory remission of 20 months. In the 14th case the Azathioprine treatment was not fully effective as a reduced dose (1 mg/kg/day) had to be given due to the side effects (cholestatic jaundice). Side effects have been observed in another case where violent vomiting occurred but neither alopecia nor decreased linear growth. In general we stopped the therapy after 24 months if there was no relapse. In conclusion our experiences so far have been encouraging. However the future will show whether our remission will result in complete cures. We suspect that Azathioprine is effective through immunosuppression. Arguments in favour of this are as follows: the disappearance of antinuclear antibodies (in about 20% of the cases), the disappearance of lymphocytes specifically adapted against colonic mucosa (identified by the method of Neth & Blaker *Klin Wschr* 48:55, 1970) and the return of complement C3 in association with clinical improvement during Azathioprine therapy.

first hour it represents about 2/3 of the total lipids, between the 100th and 200th minute it only constitutes half of the initial value.

In lipase deficiencies (congenital absence of lipase cystic fibrosis) the proportion of TG and DG is increased considerably leading to a significant reduction in the percentage of lipids solubilised. FFA and MG are concentrated in the micellar phase. In bile salt deficiencies (complete atresia of the extra hepatic bile ducts, ileal resection) the micellar phase is practically non-existent; a diminished rate of hydrolysis is also demonstrated: the importance of the interface renewal in hydrolytic process is thus underlined.

F Hanna (Columbia). *The bile acids. The digestion and absorption of different triglycerides and the effects on the absorption of calcium and magnesium.*

Bile acids role in calcium absorption is multifaceted. The absorption of vitamin D is enhanced by bile acids. This indirectly influences the synthesis of calcium carrier protein in the intestinal mucosa and is a delayed effect. The immediate effects include solubilization of the sparingly soluble calcium salts in the diet and this action explains some of the discrepancy in experimental results depending on the solubility of the calcium source in the diet. Micelle formation and the triglyceride saponification require the presence of bile acids in the intestines. Here the role of bile acids depends on the type of triglyceride in the diet. Triglycerides of unsaturated fatty acids and triglycerides of saturated fatty acids with the poorly absorbed palmitic acid in the β position favor increased calcium absorption and the free palmitic acid to a lesser extent. Stearic acid carries calcium as insoluble calcium soap in the stools. The mechanism of magnesium absorption is different and hence stool magnesium does not correlate with stool palmitic and stearic acids. On the other hand sequestration of bile acids by cholestyramine did not decrease calcium absorption in one experiment. Anionic

ABSORPTION IN RELATION TO BILE SALT METABOLISM

B Borgstrom (Lund). *Present concept of fat absorption.*

C Ricour & J Rey (Paris). *Kinetics of fat hydrolysis and micellar solubilisation in bile and lipase deficiencies.*

A duodenal perfusion was performed on 16 children with a double lumen tube. An interesterified oil mixed into a test meal was perfused as a substrate (20 mg/min/m) for 4 hours. Bile and lipase concentrations and the composition of the oil and micellar phases of the intestinal content were analysed.

In normal children the lipase concentration (300 U/D (ml) and the molar percentage of FFA (75% of the total lipids) released by hydrolysis of the substrate are constant during the perfusion whereas the proportion of FFA and MG solubilised is modified proportionately to the bile salt concentration during the

Table 1

Diagnosis	No	Coefficient of fat absorption	
		Range	Average
Malnutrition	10	87.3-98.2	93.3
Infectious hepatitis	7	94.5-98.4	96.9
Indian childhood cirrhosis	2		94.8
Congenital biliary atresia	1		38.9
Controls	10	96.7-98.7	97.9

detergent, sodium lauryl sulfate has an effect similar to bile acids on the sparingly soluble calcium salts. When bile acids are excluded from the intestinal milieu, other factors influencing calcium absorption like lactose and amino acids particularly lysine and arginine play a more important role in enhancing calcium absorption.

L. S. Prasad & C. M. Khurana (Patna, India)
Quantitative determination of faecal fat in liver disorders and malnutrition by balance studies

The aim of this study was to determine the absorption coefficient of fat on a balanced diet containing 30 to 50 g of fat per 24 hours. The method of fat estimation was that described by van de Kamer et al. Besides a normal control group, we included 10 cases of malnutrition and 10 cases of liver disease. Two of the 10 cases of malnutrition (age 1-7 years) had kwashiorkor and 8 had the marasmic type of malnutrition. One case of malnutrition presenting growth retardation showed on pilocarpine iontophoresis sweat sodium of 92 mEq/l but the coefficient of absorption of fat was as high as 97.3%. In the liver disease group (age 2 months-12 years) seven were in the recovery phase of infectious hepatitis showing enlargement of liver but without bile pigments or bile salts in the urine, 2 cases of Indian childhood cirrhosis and 1 case of congenital biliary atresia with dextrocardia and situs inversus. The results are given in Table 1.

Quantitative determination of faecal fat was also carried out in healthy dogs which were put on a diet containing 50 g of fat in the

form of butter (excluding moisture) and 2 litres of skimmed milk with sugar. After 3 days 24 hour samples of their stools were analysed for total fat (T), hydrolysed (H) and unhydrolysed fat (U) for 5 days by van de Kamer's method. The absorption coefficient of fat was calculated from the sliding mean from 3rd to 5th day's sample of stool.

After ligation of the hepatic duct and a recovery period of 5 days, the dogs were again put on a balanced diet as before and the fat excretion from 4th to 8th day was determined as mentioned above.

Following this, the fat excretion was determined while the dogs remained on the same diet but with the addition of Colibil (a syrup containing choline and bile compound with sorbitol) in a dose of 2 teaspoons thrice daily. The results are given in Table 2.

B. Strandvik & A. Norman (Stockholm)
Bile acid metabolism in infants with liver disease

The metabolism of bile acids has been studied in infants with biliary atresia (b.a.), neonatal hepatitis syndrome (n.h.s.) and other diseases with cholestasis. After intravenous administration of cholesterol 4-¹⁴C to infants with b.a. isotope was mainly excreted in urine and to a minor extent in faeces. The labelled products in faeces were neutral steroids and in urine mainly cholic and chenodeoxycholic acids. Cholic acid 24-¹⁴C was administered intramuscularly to infants with various liver diseases and the excretion of isotope in urine and faeces followed for 4 days. The nature of the labelled products and the total excretion of cholic and

Table 2 Experimental data in dogs

No	Sex	Age	Pre-operative		Post-operative	Post-operative + Colibil	Remarks
1	■	1yr	T	2.77	4.35	—	Dog died
			H	1.01	2.87	—	
			U	0.65	1.26	—	
2	M	2yrs	T	0.684	1.026	0.570	
			H	0.560	0.906	0.417	
			U	0.124	0.120	0.153	
3	F	1yr	T	1.095	0.655	0.456	
			H	0.895	0.494	0.366	
			U	0.400	0.161	0.075	
4	M	4yrs	T	0.986	1.055	0.837	
			H	0.518	0.919	0.588	
			U	0.268	0.136	0.269	

chenodeoxycholic acid were determined. In infants with b.a. n.h.s. or other diseases with cholestasis nearly all of the excreted isotope appeared in urine. Only trace amounts of labelled unconjugated cholic acid were excreted in urine. In addition to glycine and taurine conjugates other conjugates of cholic acid were excreted. No 7 α dehydroxylation of cholic acid was observed. All patients had a high daily urinary excretion of cholic and chenodeoxycholic acid. There were no differences in total urinary excretion of bile acids or in the metabolism of cholic acid between infants with b.a. n.h.s. or other diseases with cholestasis. The faecal fat excretion was increased and the absorption of vitamin A and triglycerides were impaired when the urinary bile acid excretion was increased. Improvement of the fat absorption was observed when the bile acid excretion became normal.

■ Borgström A, Nordén & ■ Ståhl (Lund)
Primary (?) bile acid deficiency in a patient
with ■ malabsorption syndrome

A 24 year-old woman has suffered from diarrhoea since birth. At 7 years coeliac disease was suspected but the diagnosis was not confirmed. In 1964 she had severe bleeding following a tooth extraction. Vitamin K deficiency but no other coagulation abnormality was found. In 1967 she appeared again with bleeding

symptoms. She had not taken vitamin K. During the last 4 years she had had increasing difficulties with her legs and could only climb a stair with the aid of her hands. Her height was 152 cm and she weighed 41 kg. She had absence of muscle reflexes and vibratory sense in both legs and Babinski's sign was present bilaterally suggesting combined degeneration of the cord but there was no evidence of vitamin B₁₂ or folate deficiency. Vitamin A treatment restored her deficient dark adaptation. Plasma tocopherol was low. There was no evidence of vitamin D malabsorption. Daily fat excretion was 40–50 g. Pancreatic lipase, amylase, trypsin and chymotrypsin were normal. The histology and disaccharidase and dipeptidase activities of the small intestinal mucosa were normal. Xylose, glucose and lactose tolerance was unaffected. X-ray examination of the intestine showed a few wide loops of doubtful significance.

Serum bilirubin, alkaline phosphatase, GOT and GPT were normal. Serum cholesterol was 266 mg with 195 mg esterified. Urinary urobilinogen was occasionally positive. The bromsophalein clearance test showed a 19% retention at 30 min and a subsequent rise to 29% at 60 min persisted for at least another hour. Cholecystography was normal. A liver biopsy showed only minor histological changes with some pigmentation of lipofuscin type. Electron microscopy showed increased smooth

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from the loop. These findings correlate with the ratio of unconjugated to conjugated bile salts found by semiquantitative thin layer chromatography in the luminal contents from each of these three areas.

References

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G W Meeuwisse

surface endoplasmic reticulum. Microvilli were short or absent. Lipofuscin like particles were found in lysosomal structures. A few bile thrombi were found in bile canaliculi.

Duodenal aspiration following a test meal was done twice. Thin layer chromatography and gas chromatography showed low content of bile acids. On stimulation with cholecystokinin *in vivo* the same deficiency was found, the total concentration of bile acids being 8.8 μ moles/ml, all conjugated and with a glycine to taurine ratio of 0.8. By measurement in faeces and urine a half life of 5.5 days of intravenously injected sodium cholate-24- 14 C was found, 25% of which was eliminated via the urine. Normally, only trace amounts will appear in the urine. A parallel study in a patient with severe primary biliary cirrhosis showed an excretion in the urine of 15% of the dose. Thus our patient was found to have a slightly reduced elimination rate of labelled cholate and a urinary excretion exceeding the excretion seen in a patient with severe parenchymatous liver disease.

The patient may suffer from a limited possibly specific defect in her bile acid metabolism. Attempts to study bile acid synthesis were made by injecting labelled cholesterol intravenously, but the radioactive dose proved to have been too small. The defect appears to have been present at birth. Chromosomal studies have shown a normal pattern. Studies of the family have included liver function and in most relatives, fine needle liver biopsies. Only one sister had had signs of liver pathology with jaundice during pregnancy. She had no signs of liver disease when examined. Four other siblings, both parents, 12 siblings of the patient's father and 5 of her mother all showed no signs of a disease resembling that found in the patient. Hence, we have no evidence that the disease represents an inherited defect. Substitution with bile acids has reduced stool frequency and the steatorrhea. At present the major complaint is walking difficulties. Muscle histology shows changes resembling those found in rabbits on a tocopherol deficient diet. We

have given the patient tocopherol orally and intramuscularly for 1½ years with no subjective or objective signs of improvement. The plasma tocopherol level has been kept normal. Recently we have started intensive treatment with vitamin B₁₂ even though there have been no signs of vitamin B₁₂ deficiency. In addition she receives vitamin A and vitamin K.

M. Gracey, Valerie Burke & A. Oshin (Birmingham) *Effect of deconjugated bile salts on intestinal sugar absorption*

Though abnormalities of fat and vitamin B₁₂ absorption are widely recognized in the 'blind loop' or contaminated small bowel syndrome, little is known of the state of carbohydrate absorption in this disorder. Our clinical observation (1) that some young babies with abnormal small intestinal flora and deconjugated bile salts have impaired monosaccharide absorption, has stimulated us to investigate experimentally the effect of bile salts on intestinal sugar absorption.

Using Semenza's (2) *in vitro* system we have found uptake of glucose and its actively transported, non-metabolized analogue arbutin (*p*-hydroxyphenyl- β -glucoside) to be impaired in the small intestine of the normal rat in the presence of 0.5 mM sodium deoxycholate, while there is no inhibition by sodium taurocholate at a concentration of 10 mM. The inhibition caused by unconjugated bile salt is reversible, and is not related to non-specific tissue damage.

We have further investigated this disturbance in rats with surgically constructed jejunal blind loops. The absorption of glucose and arbutin was studied in rats with jejunal blind loops 4-8 weeks after operation. Uptake of these substances was studied *in vitro* in the small intestine proximal to the loop, in the loop itself and in the small intestine distal to the loop. Inhibition of sugar uptake is maximal in the blind loop and is abnormal in the entire small bowel distal to the loop and is normal only in proximal small intestine more than 10 cm away

transfusion service morbus hemolyticus neonatorum, prophylactic and epidemic medical problems in blood transfusion service and consumption coagulopathies. One of the sections deals with the genetic regulation of protein synthesis and gives also aspects on the antigen structure of human immunoglobulins and haptoglobins. Special articles on the polymorphism of Gc, Hp, and other serum group systems can also be found in this section.

Another part contains communications in which different aspects of the Rh hemolytic disease are discussed: chemical analysis of amniotic fluid intra uterine blood transfusions, and above all prophylactic treatment with anti D immune globulin. The section of coagulopathies covers the theme of intravascular clotting from both experimental and clinical point of view. The clinical part contains reports dealing with etiological, diagnostic and therapeutic aspects of this really important clinical problem.

Bengt Low

M Adinolfi (ed) *Immunology and development*. Clinics in Developmental Medicine No 34. W Heinemann Medical Books Ltd London 1969. 187 pp. illus 63s.

The development of immunological competence in man is a very complicated process beginning early during fetal life. Knowledge of the immune mechanisms in primitive species and of the phylogenetic development of the immune system is essential for the understanding of the ontogenetic development. In turn, knowledge of ontogeny is very important in judging suspected states of defective immunity in infancy and childhood.

This book is mainly a review of the rapidly increasing amount of knowledge in this field but it also gives information of recent scientific results some of which are not yet published elsewhere. The book is primarily intended for paediatricians and immunologists but is undoubtedly of great general interest. For the practising clinician lacking a more profound knowledge of immunology some of the chapters are perhaps too theoretical and difficult to read while others are of greater practical value. Owing to the collected amount of knowledge and the richness in valuable references the book is clearly of interest for the paediatrician however.

The book consists of six chapters, written by different authors. The first chapter by Dr D W Talmage deals with the nature of the immunological response. The condensed account demands a great deal of the reader. The discussion on the genetic basis of immunological diversity should be of great interest for immunologists and also for geneticists. The chapter written by Adinolfi & Wood on ontogenesis of immunoglobulins and components of complement in man is clear and well written and contains a great deal of information of theoretical as well as of practical value. Clem & Leslie's report on phylogeny of immunoglobulin structure and function is of great interest and seems well justified in a

book like this. Immunological processes in mammalian reproduction are discussed by Dr W D Billington. The confusing fact that the fetus normally avoids any homograft rejection in spite of genetic dissimilarity between mother and offspring may be explained by a masking of the histocompatibility antigens of the trophoblasts by a negatively charged mucoprotein coat. Treatment with neuraminidase seems to be able to expose the underlying cell surface antigens.

The last two chapters of the book are concerned with clinical questions. Dr J R. Hobbs survey of primary immune paresis deals with clinical patterns of defective immunity in childhood. His classification of these conditions is very detailed but at the same time of practical value though some of the investigations recommended are only available in highly specialized centers. The statement that the majority of individuals with isolated IgA deficiency have symptoms does not agree quite well with recent Scandinavian findings. Whether patients suffering from ataxia telangiectasia are susceptible to infections because of IgA deficiency or because of defective cellular immunity may perhaps also be discussed. Dr R. B. McCoanell's chapter on the immunological relationship between mother and fetus deals mainly with immunization of the mother by passage of erythrocytes, leucocytes and platelets from the fetus. The theoretical basis for prevention of maternal Rh immunization by treatment with γ G anti Rh is discussed.

The book can be highly recommended to physicians interested in paediatrics and immunology.

Torsten Berg

Earl J Brewer Jr (ed) *Juvenile rheumatoid arthritis*. Vol VI. Major Problems in Clinical Pediatrics. Saunders Philadelphia, London and Toronto 1970. 231 pp. illus 25 6s.

Brewer is the head of the Arthritis Clinical Research Center Texas Children's Hospital of Houston Texas. His recently published book on Juvenile Rheumatoid Arthritis (JRA) is appreciated. JRA is little dealt with in pediatric literature though this disease is not more rare than juvenile diabetes mellitus or nephrosis. The methods for treating JRA are still unsatisfactory and the disease is crippling to a frightening extent.

Those expecting thorough discussion of immunological theories will perhaps be disappointed. Instead the book gives an excellent picture of the clinical manifestations of juvenile rheumatoid arthritis in its various forms from the fulminating type called morbus Wessler by many pediatricians, to the seemingly innocent monoarticular type. Differential diagnosis is well dealt with. The author stresses the less known complications such as pericarditis, marked leukocytosis, chronic iritis (beginning without symptoms and thus dangerous), lesions of the cervical joints with neurologic complications and local growth disturbances that may contribute to joint deformities. Radiographic changes and serology are dealt with in

BOOK REVIEWS

K. Adamsons (ed.) *Diagnosis and treatment of fetal disorders* Springer Verlag Berlin Heidelberg and New York 1969 304 pp illus DM 59 20

The establishment of prenatal medicine as a separate subspecialty in the 1960's has led to a productive integration of obstetrics, pediatrics and basic medical disciplines. This integration of disciplines has resulted in a growing concept of the fetus as a patient to whom diagnostic and therapeutic procedures could and should be offered with the same effort and intensity as with other patients. However the application of advanced techniques to the study of prenatal events has resulted in a rapidly increasing amount of information which is already difficult for the individual physician to survey.

This book gives a comprehensive view of the goals already achieved in this field and also of the future possibilities as to diagnosis and treatment of disorders affecting the human fetus in the gestational period and during the process of birth. The book is the result of an international conference held in Puerto Rico October 1967 under the auspices of the Department of Obstetrics and Gynecology Columbia University New York and participated in by a selected group of leading authorities within this new field of medicine.

Twenty-two papers are presented in the book which is divided into five sections.

The first section deals with the diagnosis of fetal conditions by means of morphologic and cytogenetic methods and gives a good review of the present concept of fetal deprivation and growth retardation and also of diagnostic possibilities offered by histologic, metabolic, enzymatic, chromosomal and other determinations in placental and fetal tissues obtained by antepartum biopsy techniques.

The second section concerns metabolic studies on the cellular growth patterns of the placenta under different conditions and on placenta and the fetal-placental unit in the synthesis and transfer of proteins, amino acids and hormones. Those papers are of great theoretical interest and give suggestions concerning the complex interrelationship between abnormal fetal growth and biochemical abnormalities in the placenta in terms of altered synthesis and content of proteins, DNA and RNA, cell number and other factors as found in maternal diabetes, maternal malnutrition and conditions of intrauterine growth failure.

The third and fourth sections deal with clinically applicable new ways of assessing and monitoring the condition of the human fetus near term or in labor, namely amniocentesis, biochemical analysis of the amniotic fluid, acid base determinations of the fetal

scalp blood and biophysical techniques for fetal electrocardiography and fetal heart rate determinations. An extensive and world wide interest is currently focused on these procedures as potential means of solving the diagnostic difficulties pertaining to fetal distress. The papers presented give as a whole a good survey of the clinical possibilities and limitations and the basic theoretical problems related to these new methods. These sections should be very informative to the individual obstetrician who attempts to adopt these procedures in his own department.

The fifth section entitled "prenatal treatment" comprises papers mainly concerned with intrauterine fetal transfusion in erythroblastosis fetalis, a method which has not met with any enthusiasm in Scandinavia. One paper however surveys the development of prevention of Rh immunization by means of passive Rh immunoglobulin, a method which recently has been introduced on a broad scale in Sweden as in several other countries. The section ends with an interesting paper on experimental enzyme inductions in fetal and newborn animals making it possible to interfere selectively with different metabolic reactions in order to study e.g. teratogenic and other effects of drugs.

Due to the book's broad approach and high quality it unquestionably holds a prominent position in current literature on prenatal medicine. The opinion of the referee is that it should be read by all obstetrician and pediatricians as well as by other interested in this important field of medicine. The book might be considered as a textbook of prenatal medicine for medical students pertaining to the resolution on medical education adopted by the International Pediatric Association and by the General Assembly of the World Congress of Obstetrics and Gynecology in New York 1970.

Lennart Jacobson

L. P. Hollander & M. Matthes (ed.) *Ergebnisse der Bluttransfusionsforschung* X. Bibliotheca Haematologica No 32 Karger Basel and New York 1969 348 pp 164 illus sFr 79

Proceedings from the 13th meeting of the Deutschen Gesellschaft für Bluttransfusion in Graz May 1968 form the 10th volume of the *Ergebnisse der Bluttransfusionsforschung*.

The proceedings comprise 41 papers most of them by invited speakers. The following subjects are covered more or less in the different sections: the polymorphism of serum groups, automation in blood

INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND ACIDOSIS ON LIPID MOBILIZATION IN THE HUMAN INFANT DURING THE FIRST TWO HOURS AFTER BIRTH

BENGT PERSSON and RAGNAR TUNELL

From the Departments of Paediatrics Karolinska Institutet St Gorans Sjukhus and Karolinska Sjukhuset Stockholm Sweden

Lipids are believed to be a minor source of energy for the human fetus (11). In accordance with this assumption are the recent findings of low concentrations of free fatty acids (FFA) in human fetal scalp blood (51) and the very low concentrations of FFA and glycerol in cord blood (29-38). The postnatal rises in plasma concentrations of FFA (9, 12, 21, 22, 29, 38, 41) and glycerol (29-38), the increases of plasma triglyceride (38) and blood ketone concentrations before the first feeding (2, 25, 26, 38, 39) and the fall in respiratory quotient (10) all indicate that the newborn infant increases lipid mobilization and oxidation of fat within the first hours after birth. Several possible explanations for this increase in lipid mobilization have been suggested (9, 12, 21, 27, 38) but the most comprehensive explanation which is based on experimental evidence (13, 14) is the increase in sympathetic tone that occurs after birth.

It is important to note that changes in the rate of mobilization of FFA from adipose tissue are not only controlled by factors which stimulate or inhibit lipolysis but also by variations in the uptake, re-esterification and possibly oxidation within adipose tissue of FFA.

Recent *in vitro* studies indicate a different regulation of these processes in the newborn human adipose tissue as compared to older infants and adults (18, 30, 31, 32, 33).

The postnatal rise in plasma FFA concentrations in newborn rabbits occurs only at an environmental temperature below 33°C (47). Systematic studies on the possible influence of the thermal environment on postnatal lipid mobilization in the human newborn infant have not been reported.

An antilipolytic effect of severe acidosis has recently been demonstrated (40, 52). Experimentally induced hypoxia in puppies inhibited lipolysis even when the animals were subjected to cold stress (3, 4). *In vitro* and *in vivo* results indicate that lactate and β -hydroxybutyrate can also inhibit lipolysis (5, 6, 7, 28).

Because of the complexity of factors affecting lipid mobilization, the lack of information concerning several of these factors and the different sampling and analytical techniques, interpretation of previous studies in the human infant on postnatal lipid metabolism is difficult.

The aim of the present study was to investigate the possible influence on postnatal lipid mobilization in the newborn human infant of labor and delivery, different environmental temperature, degree of acidosis as well as the concentrations of lactate and β -hydroxy

This study was supported by grants from the Swedish Research Council (project 668 19X 1035-03 and 871 13P 3 68-01), Karolinska Institutet Reservationsanslag and S. and J. Ford for nursing research.

special chapters. One must remember however that as a rule pathologic X ray findings appear only after the disease has lasted a few years and that rheumatoid factor is seldom present. Physiologic aspects partly of a speculative nature are given disproportionately large space compared to therapeutic exercise and physical therapy. Therapeutic exercise is presented only in the form of home treatment by parents and the methods are quite crude. The author completely omits modern physiotherapy for rheumatoid arthritis where non weight bearing exercises especially in a pool precise traction methods articulation and isometric strength training are of great importance for the prognosis. In the chapter on orthopedic treatment an attempt is made to analyze the mechanics involved in correcting knee contractures. However this analysis is far too superficial. The importance of resulting joint forces that cause cartilage destruction and infarctions when bracing and exercises are improperly performed is completely neglected.

Drug therapy is extensively treated although chloroquine is hardly given any space in spite of its being a far better anti-inflammatory drug than indomethacin. Eye complications when using chloroquine are strongly over-emphasized by the author at least according to Swedish experience.

Finally there is a short presentation of surgical methods both early procedures such as synovectomy and later reconstructive measures e.g. osteotomy.

In a patient material presented by Brewer (100 patients) 33 were functionally restored, 51 developed slight disability, 11% were severely crippled and 5 died. This is a good result compared to other materials where severe disability is reported in 20-30%. Brewer stresses the prognostic importance of early treatment at a specialist clinic with an experienced team of pediatricians, rheumatologists, orthopedic surgeons, physiatrists and physiotherapists. Those lacking such resources should refer their patients to special clinics.

Helena Svantesson

Troubles vasculaires de l'enfant et de l'adolescent
Symposium organized by the Société Française de Phlébologie. L'Expansion Paris 1970. 172 pp. illus. F 37.

At least in Sweden vascular diseases in childhood appear to be sparse. This impression is based on the reviewer's experience from a department of vascular surgery in which about a dozen children are examined

every year as contrasted against approximately 7 000 adults.

In 1967 however a questionnaire concerning the frequency of arterial and venous diseases in children was mailed to 49 experienced angiologists and pediatricians in France, Belgium, the Netherlands and some other French-speaking countries. The answers were analyzed by Dayras & Giron Paris. It turned out that angiologic diseases appeared to be more common than previously assumed. The majority of the physicians stated such cases to occupy between 1 and 2% of their practice, the upper limit was 7% by some dermatologists including angiomas in vascular diseases. Among other interesting figures may be mentioned 110 cases of the Klippel-Trenaunay syndrome, post-operative thrombosis of deep veins in 30 cases with appendectomy as the main pathogenetic agent and varicose veins amounting to 0.5-2% of phlebological practices.

This investigation including comments by several distinguished angiologists served as a basis for a symposium sponsored by the French Society of Phlebology in 1967 and is recently published to ether with several interesting contributions to pediatric angiology. May the reviewer be excused for a deep sigh: is it really necessary that printed reports from symposia so very often are published years after their original presentation? In this case the publication was three years late and it cannot be helped that in some cases part of the freshness is lost.

Several contributors deal with the problem of deep thrombosis in infancy. There are however no hints of any diagnostic or therapeutic procedure different from those used in adults. In this field one gets a remarkable demonstration of the danger of late publication since the treatment of thrombosis with streptokinase, now a routine method in angiologic clinics, is not even mentioned.

According to my personal view the most valuable paper is that by the Dutch master of phlebology H. R. van der Molen on the syndrome of Klippel-Trenaunay, i.e. cutaneous hemangioma, osseous hypertrophy and venous insufficiency with its usual complications all confined to one leg or one arm. Van der Molen presents a broad review and gives advice on the therapy. The possibility of arterio-venous anastomoses in the affected limb is clearly pointed out. It should be stressed, however, that several phlebologists particularly of the Scandinavian school prefer surgery rather than sclerotherapy in this disease.

Knut Haeger

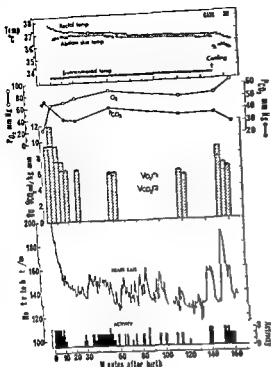
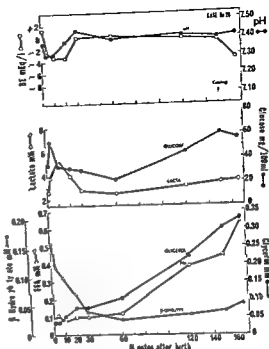


Fig 1 Sampling times and parameters measured in a normal infant (case 28 Table 1) Note the effect of cooling at 150 min



butyrate. A group of fullterm newborn infants were studied during the first 2 hours after birth in controlled environmental temperatures and simultaneous serial determinations of pulmonary gas exchange, heart rate, blood gases and acid base balance in these infants with special emphasis on the changes occurring during the first minutes after birth will be published separately.

MATERIAL AND METHODS

The nature and aim of the investigation was explained to the parents and all gave their consent.

Clinic I data on the 25 mothers and their infants are given in Table 1. The mean age of the mothers was 31 years (range 19–34 years). All mothers were healthy, their pregnancies were uncomplicated and all were delivered at term. Two mothers (cases 3 and 11) were delivered by elective caesarean section with lumbar anaesthesia because of contracted pelvis. In case 11 the anaesthesia was complicated by a transient drop in maternal systolic blood pressure to 60 mm Hg immediately before delivery. The remaining mothers had vaginal deliveries. Nitrous oxide

was not administered to any mother during delivery because the postnatal pulmonary elimination of nitrous oxide by the infants would interfere with the determination of pulmonary gas exchange (53).

Four mothers were given pudendal nerve block (0.5% Citanest® AB Astra Sweden, total 10–20 ml). Six mothers received pethidine during labor (Table 1). Twelve mothers were given intravenous oxytocin in fusion (20 IE oxytocin/1 000 ml) of a solution containing 5% fructose and 5% glucose at a rate of 30 ml/hour. The indications were slight secondary hypotonic uterine inertia (cases 13, 22 and 24), oxytocin was given only during the last hour of labor. Rh sensitization (cases 15 and 28), both infants were Rh negative and in order to avoid postmaturity (case 27).

All labors and deliveries were carefully observed and fetal heart rate was monitored intermittently using an ultrasonic technique (53). Fetal bradycardia (below 100 beats/min between contractions) was registered in 7 cases during the second stage of labor and in 5 of these infants a nuchal cord was found in one of them (case 14), the amniotic fluid was meconium stained. Nuchal cords were also found in cases 25 and 26 (Table 1). Despite the existence of these prenatal complications 24 infants were clinically normal at birth and had Apgar scores of 7 or more at 1 min and 10 at 5 min after birth. One infant

Table 1 Clinical data on mothers and infants

Analgesia																
Group	Case no	Sex	Parity	Duration of		Oxytocin admin	Pud block	Pethidine ^b		Fetal brady cardia	Mode of delivery presentation	Cord around the neck	Gest age ^c (weeks)	Birth weight (g)	Length (cm)	T _g ^d (°C)
				Labor (hours) ²	Sec stage (min)			1-3.5 hours	>3.5 hours							
I	3	♀	II	12	40		+				Caes sect		39	3 050	50	34.6
	7	♀	I	2.5	5			+			Vertex		40	4 255	55	34.8
	12	♂	III	6	20			+			Vertex		40	3 580	52	34.7
	17	♂	III	6	20			+			Occiput post		38	2 770	49	34.8
	19	♂	III	3	5						Vertex		40	3 580	52	34.7
	23	♀	I	7	50			+			Vertex		40	3 200	50	33.9
	25	♂	II	6*	5						Vertex	+	40	3 820	50	33.4
	26	♂	II	3*	10						Vertex	+	39	2 960	50	33.2
	28	♂	IV	5	5	+					Vertex		40	3 120	48	33.5
	29	♀	III	3*	5						Vertex		40	4 040	52	33.3
II	11	♂	II								Caes sect		39	2 980	49	34.7
	14	♀	I	9	10			+		+	Vertex	+	41	3 580	50	34.5
	15	♂	II	4	20			+		+	Occiput post	+	40	2 980	49	31.8
	22	♀	I	12	40			+		+	Vertex	+	40	2 900	49	33.6
	24	♂	II	14*	20			+		+	Vertex	+	41	3 890	54	33.5
	27	♂	IV	7	10			+		+	Vertex	+	42	3 680	50	33.3
	9	♀	V	3*	5						Vertex		39	3 370	51	32.0
	13	♀	II	5	10						Vertex		39	3 320	52	31.7
	16	♂	IV	4	10				+		Vertex		41	4 080	53	32.0
	18	♀	I	7*	20						Vertex		39	2 930	48	28.8
III	20	♀	III	4.5*	10						Occiput post		41	2 660	48	28.7
	21	♀	I	17*	60			+	+		Vertex		40	3 030	50	29.8
	22	♀	I	8	20			+		+	Vertex		40	3 790	53	33.8
See text	2	♀	I	8	60			+	+		Vertex		40	3 570	52	34.1
	5	♀	I	15	—			+	+		Vertex		40	3 620	51	34.0

^a Duration of labor was the time elapsing from cervical dilation of 2 cm to delivery or estimated from parturiogram (*).

^b Pethidine 50-100 mg was administered within 1-3.5 hours or more than 3.5 hours before delivery.

^c Gestational age was calculated in completed weeks from the date of the first day of the last menstruation.

^d T_g = environmental temperature.

Table 2 Comparison between umbilical venous (UV) and arterial (A) first sample drawn mean sampling time after birth was 1.4 min values for FFA glucose β hydroxybutyrate and lactate in 16 infants

		Mean \pm SD	P_1	r	P^2
FFA (mM)	UV	0.262 \pm 0.072	<0.01	0.71	<0.01
	A	0.204 \pm 0.082			
Glycerol (mM)	UV	0.0735 \pm 0.0192	ns	0.57	<0.05
	A	0.0691 \pm 0.074			
β -Hydroxybutyrate (mM)	UV	0.314 \pm 0.191	<0.001	0.92	<0.001
	A	0.200 \pm 0.133			
Lactate (mM)	UV	3.85 \pm 1.35	<0.05	0.84	<0.001
	A	4.57 \pm 2.01			

P_1 = degree of significance between UV and A values using paired t test

P^2 = degree of significance of the correlation coefficient (r) between UV and A values

trisodium citrate) drawn at 1-10 min of age. In an additional 6 cases lipoprotein lipase activity was determined at the end of the study during the third hour after birth before and 10 min following a single intravenous dose of heparin (500 units in 10 ml of 0.15 M sodium chloride solution). The samples were also analyzed for glucose FFA glycerol and β hydroxybutyrate. Plasma post heparin lipoprotein lipase activity was assayed *in vitro* according to the method described by Bobberg & Carlson (8) except that FFA was analyzed by a micromethod (23).

Study protocol

Immediately after birth the upper airways of the infant were carefully cleared of mucus by suction. One group of infants, predetermined for observations at a high environmental temperature were dried with warm (39°C) towels and were kept below a radiant heat source. Another group of infants predetermined for observations at a lower environmental temperature were dried with towels (22°C) and were kept in room temperature during the first minutes after birth. Within the first minutes after birth the following procedures were performed:

1. A catheter was inserted through an umbilical artery into the abdominal aorta and the first blood sample was drawn. The umbilical vein was punctured and a blood sample was drawn. The cord was clamped 1 min after birth.

Simultaneously a face mask was applied and gas collection for determination of the pulmonary gas exchange was begun (53).

3. An electrode was attached to the infant's chest and recording of heart rate was started.

4. A thermocouple was placed 4-5 cm deep into the rectum for rectal temperature monitoring.

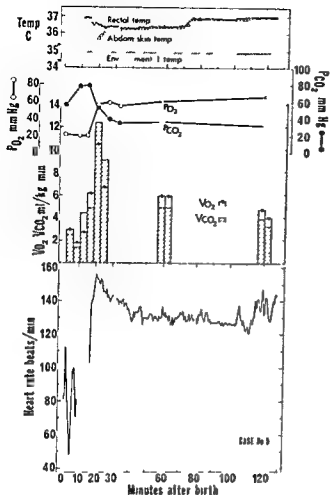
The above procedures were usually completed within 4-5 min of age and the baby was thereafter transferred to a thermoccontrolled chamber (53). The baby was kept in the chamber during the first 2 hours after birth. The mean environmental temperature of the group of infants who were predetermined

for observation at a high environmental temperature was 34.0°C (range 33.2-34.7°C). In the other group of infants which was studied at a lower environmental temperature the mean temperature was 30.5°C (range 28.7-32.1°C). Skin temperatures were recorded at six different sites at 20 min intervals. Rectal and abdominal skin temperatures three different environmental temperatures and heart rate were continuously recorded (53). Changes in motor activity were subjectively evaluated according to a four point scale: 0 = infant asleep, no movement of limbs; 1 = infant awake, movement of one limb; 2 = infant awake, movement of more than one limb; 3 = infant crying, all limbs moving. The mean activity during each 4-minute period of gas collection was calculated.

Clinical observations during labor and delivery time of birth condition of the baby, motor activity and the times of samplings were tape recorded. Blood samples were drawn at predetermined intervals during the first 2 hours after birth for subsequent determination of blood gases, acid base balance, glucose, FFA, glycerol, β hydroxybutyrate and lactate. Determination of the pulmonary gas exchange was performed continuously during the first 20 min after birth in successive 4 min intervals and during two 4 min intervals each at 60 and 120 min of age. The different parameters recorded in relation to time are illustrated by a typical case in Fig. 1.

RESULTS

Simultaneous umbilical venous and arterial blood samples were obtained in 16 infants (Table 2). There were significant correlations between umbilical venous and arterial concentrations for FFA, glycerol, β hydroxybutyrate and lactate. The umbilical venous concentrations for FFA and β hydroxybutyrate were significantly higher than in arterial blood. The



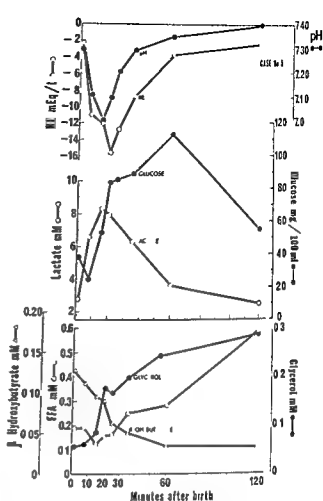
a

Fig 2 Postnatal asphyxia (case 5 Table 1) Uncomplicated term delivery pethidine was given 15 and 25 hours before delivery (100 100 75 mg) Apgar core 5 at 1 min No fetal bradycardia was registered and initial values for pH P_{CO_2} and BE

(case 5) developed postnatal asphyxia (see legend to Fig 2) All infants had birth weights and lengths within the normal range for their sex and gestational age (49)

Blood collection and analytical procedures

A 90 cm 5 French polyvinylchloride catheter was used and blood samples (2 ml each) were taken in heparinized plastic syringes at predetermined intervals during the first 2 hours of life (Fig 1) The total amount of blood drawn from each infant never exceeded 18 ml A portion of each blood sample was immediately transferred to a gastight glass syringe and stored in ice for later measurement of pH P_{O_2} and P_{CO_2} (30) The remaining blood was placed in chilled plastic tubes and stored on ice Within 60 sec after sampling a 200 μ l aliquot of the blood was transferred to 10 ml of 0.6 M ice cold perchloric acid for lactic acid determination (45) After centrifugation at 4 °C of the remaining blood plasma was separated and frozen at -20 °C until analyzed in



b

were normal Irregular shallow breathing after birth was associated with very low pulmonary gas exchange values and bradycardia After a period of intensive crying normalization of pulmonary gas exchange and blood gases occurred

duplicate for glucose (50) FFA (23) glycerol (24) and β hydroxybutyrate (39) The analytical errors were for lactate ± 2.4 glucose ± 4.1 FFA ± 5.5 glycerol ± 2.2 and β hydroxybutyrate ± 1.9

Between sampling the volume of the catheter (0.75 ml) was filled with 0.15 M sodium chloride solution The sampling procedure was as follows the sodium chloride solution was withdrawn the catheter was repeatedly flushed with arterial blood 2.5 ml of blood was withdrawn and saved a 2.0 ml sample was taken the 2.5 ml blood was re injected and the volume of the catheter was refilled with sodium chloride solution

In 2 cases numbers 1 and 2 minute amounts of a dilute heparin 0.15 M sodium chloride solution (Heparin AB Vitrum Stockholm 50 units/ml sodium chloride solution) were used to fill the catheters between samplings In these 2 cases lipoprotein lipase activity was determined on samples (0.9 ml of blood collected in a syringe containing 0.1 ml 0.1 M

0.65 mM to 0.377 and 0.90 mM respectively. The individual concentrations for lipoprotein lipase activity were significantly correlated to the individual increases in FFA concentrations ($r=0.81$ $n=6$ $p<0.001$). No significant change occurred in glucose concentrations.

Results obtained in case 5 who developed postnatal asphyxia are given in Fig 2.

The complete results of changes in pulmonary gas exchange, blood gases and acid base balance, skin and rectal temperatures, heart rate and motor activity in these infants will be published separately (53).

Influence of prenatal history

The possible influence of fetal distress, duration of labor and analgesia on the pattern of postnatal changes of FFA and glycerol were analyzed.

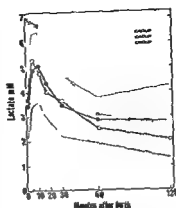


Fig 4 Mean lactate concentrations for the different groups. The shaded area represents ± 1 SD for group I.

Fetal distress. Ten infants without signs of prenatal complications were grouped together (group I, Table 1). All five infants with a history of fetal bradycardia during the second stage of labor and one infant delivered by caesarean section which was complicated by a drop in maternal blood pressure were grouped together (group II, Table 1). Groups I and II were studied in similar environmental temperatures (Table 1).

Group II had a significantly higher degree of acidosis during the first minutes after birth as compared to group I. The mean levels for pH were 7.15 and 7.30 for base excess -9.6 and -2.6 mEq/l and for P_{CO_2} 69 and 54 mm Hg in groups II and I respectively. The higher degree of acidosis in group II as compared to group I corresponded to a statistically higher

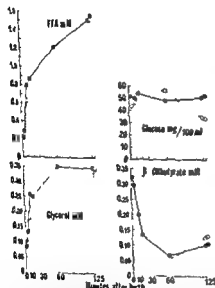


Fig 3 Plasma concentrations for FFA, glycerol, glucose and β -hydroxybutyrate in two infants (cases 1 and 2, Table 1). The catheters used for blood sampling had been flushed with a dilute heparin saline solution. Case 1 (●) Developed fetal bradycardia late in second stage of labor. At 1 min after birth there was severe acidosis with pH 7.0, P_{CO_2} 95 mm Hg, BE -17 mEq/l and blood lactate 8 mM. Subsequent clinical course uneventful. Case 2 (○) Term delivery (Table 1). Apgar scores 8 and 10 at 1 and 5 min. Blood lactate 3.97 mM at 4 min. Clinical course uneventful.

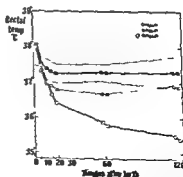


Fig 5 Mean rectal temperatures for the different groups. Shaded area represents ± 1 SD for group I.

Table 3 Mean values \pm SD for sampling times and plasma levels of glucose, FFA glycerol, β hydroxybutyrate and lactate in 22 infants (groups I + II + III)

Parameter	Sampling times minutes after birth						
	1 \pm 0.6	5 \pm 0.8	10 \pm 1.0	17 \pm 2.4	30 \pm 0.9	60 \pm 1.0	120 \pm 1.5
FFA (mM)	0.184 \pm 0.074 (19)	0.204 \pm 0.083 (20)	0.202 \pm 0.090 (20)	0.289 \pm 0.186 (22)**	0.294 \pm 0.199 (22)*	0.547 \pm 0.509 (22)**	0.757 \pm 0.383 (22)***
Glycerol (mM)	0.0742 \pm 0.0336 (20)	0.0898 \pm 0.0390 (19)**	0.1135 \pm 0.0553 (20)***	0.1488 \pm 0.0678 (22)***	0.1773 \pm 0.0755 (22)***	0.2421 \pm 0.1172 (22)***	0.2782 \pm 0.0979 (22)***
Glucose (mg/100 ml)	59.4 \pm 26.1 (19)	63.1 \pm 28.1 (22)	64.2 \pm 22.3 (22)	69.3 \pm 30.8 (22)*	58.0 \pm 23.3 (21)	56.5 \pm 22.9 (20)**	59.0 \pm 14.5 (21)
β Hydroxybutyrate (mM)	0.1758 \pm 0.1333 (19)	0.1445 \pm 0.1085 (19)***	0.1098 \pm 0.0754 (20)***	0.0879 \pm 0.0624 (22)***	0.0642 \pm 0.0393 (22)***	0.0643 \pm 0.0659 (22)***	0.0742 \pm 0.0673 (21)***
Lactate (mM)	4.55 \pm 2.06 (20)	5.74 \pm 1.94 (21)***	5.52 \pm 1.73 (22)***	4.79 \pm 1.55 (22)	3.88 \pm 1.24 (22)	2.83 \pm 0.93 (22)***	2.52 \pm 1.29 (22)***

Figures within parenthesis denote number of observations

Significant difference between values at 1 min and values at subsequent sampling times using the paired *t* test are given as * - $p < 0.05$ ** - $p < 0.01$ *** - $p < 0.001$

arterial concentrations for lactate were higher than those in venous blood

Mean values \pm standard deviations for sampling times and for arterial concentrations of FFA glycerol β hydroxybutyrate and lactate obtained in 22 infants during the first 2 hours after birth are given in Table 3. The degree of statistical differences (using the paired *t* test) between concentrations at 1 min and all subsequent concentrations are given in Table 3. A significant rise in plasma FFA-concentrations was found at 17 min and thereafter there was a progressive increase. A significant rise in plasma glycerol concentration was seen already at 5 min and was followed by a progressive increase. The glucose concentrations remained essentially unchanged during the observation period. The mean concentrations of β -hydroxybutyrate showed an initial rapid decrease with time. After a significant increase in the mean lactate concentration between 1 and 5 min, there was a progressive decrease with time.

The logarithms of the individual concentrations for lactate and β hydroxybutyrate were

plotted against time and from the extrapolated straight line formed the half times (*t*_{1/2}) were graphically determined. The mean *t*_{1/2} values \pm standard deviations for lactate and β hydroxybutyrate were 36.1 ± 19.6 ($n=15$) and 19.1 ± 11.2 ($n=20$) minutes respectively. No relation was found between the highest level of β hydroxybutyrate or lactate and the corresponding rise in FFA or glycerol between 1 and 120 min.

Individual concentrations for FFA glycerol, β hydroxybutyrate and glucose in the 2 cases (1 and 2) who received heparin saline solution are given in Fig. 3. A distinct increase in concentrations of glycerol and FFA was already seen at 5 min. Lipoprotein lipase activities at 120 min were 0.045 and 0.036 μ moles FFA per minute and ml plasma in case 1 and 2 respectively.

In six infants the mean lipoprotein lipase activity increased from 0 at 120 min to 0.084 μ moles FFA per minute and ml plasma 10 min following heparin administration. The mean concentrations for glycerol and FFA increased significantly (paired *t* test) from 0.343 and

0.65 mM to 0.377 and 0.90 mM respectively. The individual concentrations for lipoprotein lipase activity were significantly correlated to the individual increases in FFA concentrations ($r=0.81$ $n=6$ $p<0.001$). No significant change occurred in glucose concentrations.

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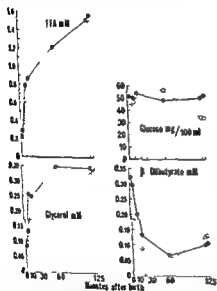


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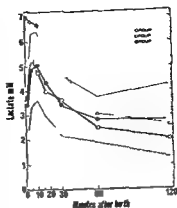


Fig. 4 Mean lactate concentrations for the different groups. The shaded area represents ± 1 SD for group I.

Fetal distress. Ten infants without signs of prenatal complications were grouped together (group I, Table 1). All five infants with a history of fetal bradycardia during the second stage of labor and one infant delivered by caesarean section which was complicated by a drop in maternal blood pressure were grouped together (group II, Table 1). Groups I and II were studied in similar environmental temperatures (Table 1).

Group II had a significantly higher degree of acidosis during the first minutes after birth as compared to group I. The mean levels for pH were 7.15 and 7.30 for base excess -9.6 and -2.6 mEq/l and for P_{CO_2} 69 and 54 mm Hg in groups II and I respectively. The higher degree of acidosis in group II as compared to group I corresponded to a statistically higher

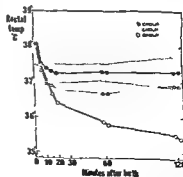


Fig. 5 Mean rectal temperatures for the different groups. Shaded area represents ± 1 SD for group I.

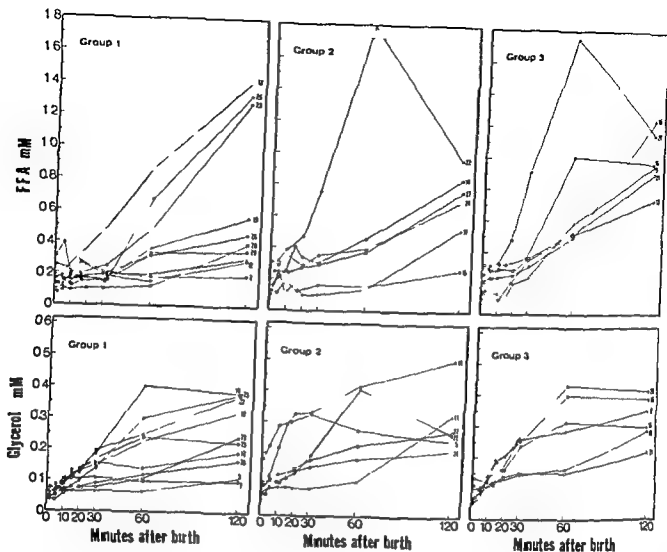


Fig 6 Individual plasma concentrations of FFA and glycerol in the groups I, II and III (from left to right)

mean lactate concentration during the first 30 min after birth (Fig 4). Group II had significantly lower mean rectal temperatures during the first 60 min after birth with the lowest mean level at 60 min (Fig 5). During the initial minutes after birth group II also had a somewhat lower mean heart rate as compared to group I (53). The mean levels and the pattern of changes in postnatal pulmonary gas exchange were similar in both groups (53).

No definite difference in the initial levels and the patterns of changes of FFA were detectable when comparing the two groups (Fig 6). The initial glycerol concentrations were slightly higher in group II at 1 and 5 min ($p < 0.05$) and the slope of the rises were steeper during the first 30 min (Fig 6). A greater

variation in the increase in glycerol concentration was seen in group I. The mean concentrations for glucose and β -hydroxybutyrate were not significantly different in the two groups (Figs 7 and 8).

The influence of postnatal asphyxia on concentrations of FFA and glycerol are illustrated in Fig 2. The pattern of changes in FFA and glycerol concentrations did not differ from those seen in groups I and II.

Duration of labor. The highest initial concentrations for β -hydroxybutyrate were found in infants of primigravidae. There was a positive correlation between the duration of labor in hours and the arterial concentrations (at 1 min) of β -hydroxybutyrate ($r = 0.80$, $n = 19$, $p < 0.001$) and FFA ($r = 0.70$, $n = 23$, $p <$

0.001) In contrast no such relationship existed between duration of the 2nd stage of labor in minutes and the concentrations of FFA and β hydroxybutyrate. No relation was found between the rise in concentration of glycerol or FFA (between 1-120 min) and the duration of labor or second stage.

Analgesia Pethidine administration to the mothers during labor was not accompanied by demonstrable differences in initial concentrations or patterns of changes for FFA or glycerol.

Influence of environmental temperature

Six infants were studied at a low environmental temperature (group III Table 1) and their results have been compared with those in 10 infants which were studied at a high environmental temperature (group I Table 1). The mean values for rectal temperatures in group III were significantly lower already at 10 min after birth and the lowest recorded mean rectal temperature was 35.4°C at 120 min (Fig. 5). During the observation period group III tended to have higher values for motor activity and lower values for P_{50} (53). The two groups were similar in the following respects: prenatal history, duration of labor, oxygen uptake and carbon dioxide elimination, and heart rate (53).

The mean concentrations for β hydroxybutyrate, lactate and glucose were not significantly different in the two groups (Figs. 4, 8 and 7).

In group III there was an earlier and more

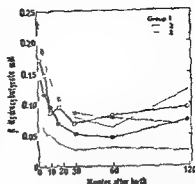


Fig. 8. Mean plasma concentrations of β hydroxybutyrate in the different groups. Shaded area represents ± 1 SD for group I.

consistent rise in plasma glycerol and FFA concentrations than in group I.

However, when the two groups were combined, no relationship was found between the individual values for environmental temperatures and the rises in FFA or glycerol between 1 and 120 min or between 1 and 30 min.

Relation between plasma FFA or glycerol and other parameters

Glucose. No correlation was found between paired concentrations for glucose and FFA or glycerol concentrations in the total material. When the lowest glucose concentration in each infant found at 30 or 60 or 120 min was related to the FFA-concentrations at 120 min, no correlation was found. Five infants had one or more glucose concentrations equal to or below 30 mg/100 ml. When comparing the increment rises in glycerol or FFA between 1 and 120 min between these 5 infants and the remaining 17, no significant differences were found.

Motor activity. A significant relationship was found between the degree of motor activity and oxygen uptake (53). The rises in FFA or glycerol concentrations (1-120 min) however were not related to the individual means of motor activity for 1-20 or 60 and 120 min.

Respiratory exchanges ratio ($R = V_{CO_2}/V_{O_2}$). The R values were significantly related to the



Fig. 7. Mean plasma concentrations of glucose in the different groups. Shaded area represents ± 1 SD for group I.

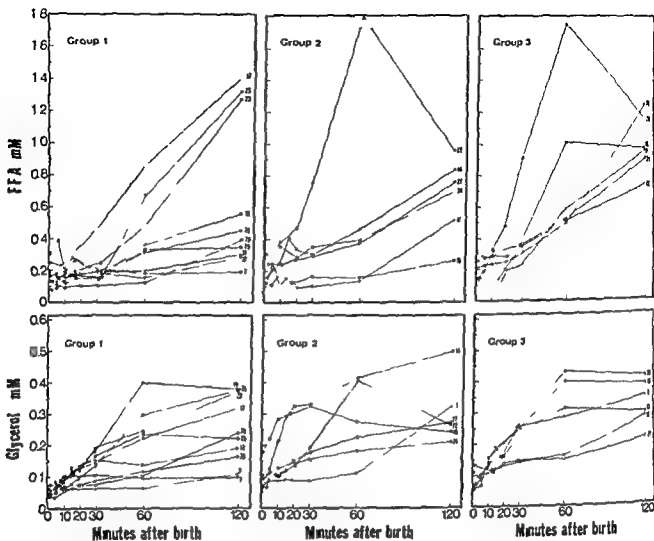


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half hour after birth FFA is either oxidized or re esterified within adipose tissue

In 21-35 day-old anaesthetized puppies experimentally induced hypoxemia resulted in acidosis and decreased plasma concentrations of FFA and glycerol (3). However the P_{50} values of the animals were low and thus the experimental situation differed from neonatal asphyxia which is characterized by stagnant hypoxia and hypercapnic acidosis. Unchanged as well as rising concentrations of plasma FFA have been reported in anaesthetized dogs following hypoxia and hypercapnia (15, 16, 36, 46). On the other hand isolated hypercapnic acidosis in anaesthetized dogs inhibited noradrenaline induced lipolysis (40). The postnatal rise in plasma FFA was inhibited in newborn lambs by severe fetal distress and in newborn rabbits by breathing 100% nitrogen (13, 47).

In the present study signs of fetal distress accompanied by acidosis at birth did not significantly influence the postnatal patterns of changes of FFA and glycerol concentrations. In the distressed group (group II) the rise in glycerol concentrations tended to be more pronounced during the first minutes as compared to those in the control group (group I). A distinct rise in plasma glycerol concentration was also seen in an infant during a period of postnatal asphyxia (see case 5, Fig. 2). The finding of a lack of inhibition of lipolysis during asphyxia in the newborn human is in contrast to most of the above mentioned experimental results. These discrepancies could be due to differences in the severity of distress, age or species variations. Interpretation of the results obtained in the dog experiments as compared to the present findings is further complicated by the use of anaesthesia in those studies since pentobarbital has been shown to influence the arterial concentrations of FFA (19).

In a pilot study where the infants were handled in the same way and with the same equipment for thermoregulation it was shown that if the environmental temperature was increased

above 34.5°C a rise in rectal temperature towards overheating occurred within the first 30 min after birth. Since the route for heat elimination of the fetus is via the placenta, the rectal and skin temperatures at the moment of birth are equal and approximately 38°C (54). Following birth a temperature gradient of approximately 2°C has to be established between the environment and skin to allow for the necessary heat elimination (1). The mean rectal temperature in group III (mean T_{re} 30°C) decreased to 35.4°C at 120 min which is comparable to the rectal temperatures reported in infants who have received routine nursery care (35). It has been reported that infants exposed to a low environmental temperature (room temperature) have higher blood lactate levels and a more pronounced acidosis during the first hours after the birth as compared to infants kept in incubators at a temperature between 32-33°C (17). The moderate difference in thermal stimulation between group I (mean T_{re} 34.2°C) and III in the present study did not result in any differences in blood glucose levels or rate of elimination of postnatal acidosis and lactate. This was in good agreement with a recent study using a similar sampling technique and environmental temperature conditions (48).

Lipolysis and lipid mobilization as indirectly reflected by the rises in glycerol and FFA concentrations occurred irrespective of the ambient temperature chosen but these processes seemed to be more pronounced in the thermally stimulated group (group III).

It has been suggested that the decrease in blood glucose concentration after birth could be one explanation for the postnatal increase in lipid mobilization. Reported findings of an inverse correlation between blood glucose and plasma FFA concentrations could be taken as evidence for this hypothesis (9, 12). No such relationship however has been found in other studies (38) or in the present investigation. In addition the rising glycerol concentrations starting already within the first minutes after birth suggest that lack of available glucose is

Table 4 Correlation between FFA and glycerol values in the different groups (see text) at different times after birth

	Age in minutes					
	1-17			30-120		
	r	n	p	r	n	p
Group I	0.38	34	<0.05	0.55	30	<0.001
Group II	0.32	39	ns	0.72	37	<0.001
Group III	0.20	24	ns	0.70	20	<0.001
Group I+II+III	0.44	97	<0.001	0.67	87	<0.001

r = correlation coefficient

n = number of observations

p = degree of statistical significance

degree of acidosis (53). At 120 min after birth when the acidosis had been eliminated in all infants, the mean R-values \pm SD for group I, II and III were 0.88 ± 0.065 , 0.85 ± 0.054 and 0.77 ± 0.076 respectively. An inverse correlation was found between the rise in FFA (between 1-120 min) and the R-value measured at 120 min ($r = 0.47$, $n = 24$, $p < 0.05$).

Relation between FFA and glycerol The relationship between FFA and glycerol concentrations was calculated during two time intervals between 1 and 17 min and between 30 and 120 min (Table 4). A relationship between FFA and glycerol concentrations in all groups was found only during the latter time interval.

DISCUSSION

The importance of not using heparin solutions for catheter patency is illustrated by the present results, confirming previous reports that heparin activated lipoprotein lipase in the newborn human (20). Furthermore, minute amounts of heparin induce significant changes in plasma concentrations of FFA and glycerol.

The differences in reported mean cord blood FFA concentrations, ranging between 0.1 to 0.6 mM in different studies (9, 12, 21, 22, 29, 38, 42, 51), probably reflect systematic differences in analytical techniques.

The results of previous studies on the influence of fetal distress during labor on FFA concentrations in umbilical cord blood as conflicting and both elevated and low levels have been reported (21, 41, 42, 51). The arterial FFA concentrations at 1 min in the present study were not significantly different between infants with or without signs of fetal distress during labor. In accordance with recent reports (44) we found slightly higher initial glycerol concentrations in the group with signs of fetal distress (group II). In addition, both initial FFA and β -hydroxybutyrate concentrations were significantly related to the duration of labor.

Our finding of no significant arterio-venous difference for glycerol concentrations in cord blood was in agreement with previously reported results (44). In contrast to one (43) but in agreement with other reports (9, 17), were the higher concentrations of FFA in umbilical venous rather than arterial blood. Unlike these studies however, this arterio-venous difference was significant, but only when the paired *t* test was used. Since concentrations of some parameters measured in cord blood samples seem to be only representative for the situation at birth being influenced by the process of labor and delivery, reported estimations of fetal energy metabolism based on cord arterio-venous differences (43) must be interpreted with extreme caution.

The results of the present study clearly indicate that almost immediately after birth an increase in lipolysis occurs as evidenced by the rapid rise in glycerol concentrations. In contrast the rise in FFA concentrations was generally delayed until between 30 and 120 min after birth. The initial low concentrations of FFA indicate a suppressed lipid mobilization. In agreement with this finding are recent *in vitro* studies on isolated fat cells from newborn human infants which have shown a high release of glycerol and a low outflow of FFA to the medium as compared to adipose tissue from older infants and adults (34). Our results would therefore indicate that within the first

been eliminated an inverse correlation was found between the rise in FFA from birth to 120 min and the respiratory exchange ratio (V_{CO_2}/V_{O_2}).

The glucose concentrations were related neither to the FFA nor to the glycerol concentrations. The rate of elimination of lactate and β -hydroxybutyrate was not influenced by environmental temperature or acidosis. Minute amounts of administered heparin caused an increased rise in FFA and glycerol concentrations which were associated with the appearance of lipoprotein lipase activity.

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■ very unlikely explanation for the increase in lipolysis

During labor the maternal concentrations of blood ketones increase and at delivery a significant relationship exists between maternal venous and cord blood concentrations (37, 39, 48). In agreement with previous studies are the present findings of a higher concentration of β hydroxybutyrate in umbilical venous than in arterial blood and the significant correlation between venous and arterial concentrations (37, 43). The highest concentrations of β hydroxybutyrate were found at 1 min after birth but they did not reach the reported concentrations known to inhibit lipolysis (6).

No relationship was found between concentrations of β -hydroxybutyrate and postnatal changes in FFA and glycerol concentrations. The hypothesis that the cord concentrations of ketones are mainly determined by the ketone body infusion from the mother to the fetus and not by the ketone body production by the fetus is supported by the present findings of a very rapid postnatal elimination rate of β -hydroxybutyrate. This rapid elimination of β -hydroxybutyrate was found in all infants irrespective of their condition at birth and their environmental temperatures.

During the first hour after birth, the respiratory exchange ratio primarily reflects the elimination of combined acidosis (53). It is thus impossible to draw any conclusions from the values of respiratory exchange ratio (R) concerning tissue metabolic respiratory quotient (RQ) during this time period. At 120 min after birth the arterial blood pH and lactate values were normalized and at this time R values could represent RQ. The findings of an inverse relation between the rise in FFA during the first 2 hours after birth and the R values at 120 min support this view. The significantly lower R-values at 120 min present in group III (T_E 30.5°C) as compared to group I (T_E 34.0°C) might indicate differences in the relative degree of oxidation of fat between these two groups of infants. However, the short registration periods (4+4 min) for the determi-

nations of R values do not allow any definite conclusions concerning the possible influence of different ambient temperatures on the degree of oxidation of fat in the newborn infant.

Although various factors (27,38) may influence lipolysis and lipid mobilization in the newborn, the strikingly rapid rise in glycerol concentrations during the first minutes after birth suggest that during this period the dominant factor controlling lipolysis is an increase in sympathetic nervous activity. In the normal fullterm infants of this study, the results furthermore suggest that the effect of environmental temperature and acidosis on the increase in lipolysis were marginal. The lack of an inhibiting influence on lipolysis and lipid mobilization of these factors is probably of great importance for the energy metabolism of the newborn.

SUMMARY

Arterial concentrations of glycerol, FFA, glucose, lactate and β hydroxybutyrate were serially measured during the first two hours after birth in normal fullterm infants in a thermally controlled environment. Blood gas tensions, acid base balance, pulmonary gas exchange, motor activity and heart rate were also determined. A detailed report of these data will be published separately. In 22 infants the glycerol concentrations showed a rapid immediate increase after birth whereas the rises in FFA concentrations were delayed until between 30 and 120 minutes, indicating a prompt increase in lipolysis and a suppression of lipid mobilization during the first half hour after birth. This suppression might be explained by a high rate of re-esterification or oxidation of FFA within adipose tissue. The influence of environmental temperature (28.7-34.8°C) and degree of acidosis on the pattern of changes in FFA and glycerol were only marginal. No inhibition of lipolysis and lipid mobilization was shown in an infant who developed postnatal asphyxia.

At 120 min after birth, when acidosis had

THE DIAGNOSIS OF TAY SACHS DISEASE¹

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Tay Sachs disease is an autosomal recessive inherited disorder characterized by widespread deposits of a specific ganglioside G_M in the neurons but also to some extent in the glial cells. Earlier work in this laboratory showed that this ganglioside differs from the major brain gangliosides by its carbohydrate moiety in that it consists of only three neutral sugar moieties leaving *N*-acetylgalactosamine in end position of the chain (25). A storage of this ganglioside was later demonstrated in some visceral organs (3). On the basis of this finding a generalized defect of a β -*N*-acetyl galactosaminidase degrading the G_M -ganglioside was predicted as the enzymatic failure in Tay Sachs disease (3).

Recent investigations on hexosaminidases in mammalian tissues (1, 18) have produced new information of importance for the further study of the enzymatic lesion in Tay Sachs disease. The hexosaminidases of normal mammalian tissues can be subfractionated into two major fractions A and B by electrophoresis, ultracentrifugation or column chromatography. Okada & O'Brien (15) who used starch gel electrophoresis showed that the hexosaminidase fraction A was missing in brain, liver, kidney, skin and white blood cells in patients with classical Tay Sachs disease. Recent research has also produced evidence

for more than one genetic form of a given gangliosidosis with a different enzymatic lesion (2, 21, 31).

The diagnosis of a ganglioside storage disease such as Tay-Sachs disease thus requires the identification of the accumulated substance and characterization of the enzymatic failure. The aim of the present study was to find a tissue suitable for determination of the ganglioside deposit in biopsy specimens and to develop a simple method for analysis of the hexosaminidase pattern.

MATERIALS

Tissues were available from a 3-year-old girl and a 4-year-old boy who had died from Tay Sachs disease. Control tissues were obtained from 20 subjects who had died of diseases not involving the central nervous system. Tissue was also available from one patient with globoid cell leukodystrophy (GCLD) from one patient with late infantile metachromatic leukodystrophy (MLD) from two patients with juvenile amaurotic idiocy (JAI) and from one patient with multiple sclerosis (MS).

A detailed case report of the 4-year-old boy with Tay Sachs disease has been published earlier (3). The other patient who died from Tay Sachs disease at the age of three years also belonged to a non-Jewish family with no earlier family history of the disease. The first symptoms of weakness and delayed motor development were noticed at the age of 6-7 months. The disease ran a characteristic course and from the age of 6 months the child assumed a permanent posture of "decerebrate rigidity". She died at 36 months from aspiration pneumonia.

METHODS

Lipid determinations. Total lipids were extracted with chloroform-methanol (1:1 v/v) and phospholipids

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Table 2 Fractionation of glycosidase activity by ultracentrifugation with a patient with Tay-Sachs disease and a control

Tissue samples for the analyses were taken from the cortex of frontal lobe. The brains were stored at -20° for 9 months before analysis. Ultracentrifugation and assay of glycosidase activity was performed as described in "Methods". The enzyme activities are given under A as μ moles *p*-nitrophenol released per hour per mg enzyme protein and under B as percent of homogenate activity.

Brain tissue	β -glucosidase		β -galactosidase lactate pH 3.6		β -N acetylgalactosaminidase		β -N acetylglucosaminidase	
	A	B	A	B	A	B	A	B
Tay Sachs patient (3.0 y)								
Homogenate	168	100	88	100	305	100	1732	100
100 000 g, sediment	213	92	62	51	387	100	2373	99
100 000 \times g, supernatant	33	2	214	29	28	1	257	2
Control (3.5 y)								
Homogenate	36	100	44	100	47	100	500	100
100 000 g, sediment	29	67	26	49	22	38	234	38
100 000 \times g, supernatant	8	0	117	39	214	65	2248	64

of that in liver. The ganglioside pattern was more complex and only about 75% of the total amount of gangliosides could be identified. G_{M1} -NAN constituted 33-50% of total ganglioside-NAN.

A typical nerve ganglioside pattern was found in the lipid extracts of the muscular and mucosal layers of the rectum. G_{M1} constituted 80% and G_{M1} and G_{D1} predominated among the other gangliosides. The ganglioside concentration was about three times higher in the muscular layer than in the mucosa.

In nervous tissue from the two subjects with Tay Sachs disease one oligohexoside, asialo-ganglioside G_{A2} was demonstrated in appreciable amounts. Its concentration in the two subjects was 3% and 6% respectively of that of G_{A1} .

The hexosamine-containing glycolipids were estimated in the spleen, liver and kidneys of controls and in the two cases of Tay Sachs disease. The asialogangliosides G_{A1} and G_{A2} could not be demonstrated in these organs but the major glycolipid had the same composition and migration rate at chromatography as globoside. Its concentration was 1140-80 μ moles/g fresh tissue in the two cases of Tay Sachs disease.

Glycosidase activities in brain tissue. The activities of β -glucosidase, β -galactosidase, β -N

acetylgalactosaminidase and β -N acetylglucosaminidase were assayed in the brain tissue from the 3-year-old girl with Tay Sachs disease. To estimate the effect of long storage of the brain material on the enzyme activities, tissue samples were assayed fresh and after storage at -20° . Storage caused a decrease in glucosidase activity but an increase in hexosaminidase activity. The changes did not however essentially affect the profile of glycosidase activities of the control and pathological material. Similar enzyme activities were obtained with 0.32 M sucrose and distilled water as homogenization medium for the tissue.

The *p*-nitrophenyl glycosidase activities in the cerebral cortex of the 3-year-old girl with Tay Sachs disease were 5-10 times those of the control material (14) with the exception of β -galactosidase (assayed at pH 3.6) which was only moderately increased (Table 2). The glycosidase activities in white matter were about 10 times higher in the girl with Tay Sachs disease than in the control material except for β -galactosidase (assayed at pH 5.0) which was only increased 5 times.

The cerebral cortical glycosidase activities were not increased in the patients with GCLD, MLD, JAL and MS. In GCLD and MS the glycosidase profile was fairly similar to that

Table 1 Concentration of gangliosides and phospholipids in various organs of two patients with Tay-Sachs disease

The figures are expressed in μ moles/g fresh tissue weight. Figures in parentheses give the number of controls analysed

Tissue	Phospholipids			Gangliosides		
	Normals (1-5 y)	K K (3 y)	J A (4 y)	Controls (1-5 y)	K K (3 y)	J A (4 y)
Cerebrum frontal cortex	44 (6)	30	21	1.5 (6)	12.1	5.6
Cerebrum frontal white matter	98 (6)	36	17	0.64 (6)	5.9	4.3
Cerebellum cortex	39 (3)	33	23	1.2 (3)	6.6	6.5
Spinal cord	102 (2)	81	68	0.45 (2)	2.8	3.8
Spinal root	100 (1)	91	59	0.20 (1)	1.2	1.3
Adrenal		40			1.4	
Liver	26 (4)	29	41	0.02 (2)	1.4	0.6
Spleen	16 (4)	22	23	0.12 (2)	1.2	0.7
Lung		15			0.1	
Kidney	19 (4)	19			0.2	
Ovary		15		0.05 (2)	0.3	
Rectum mucosa		14			0.3	
Rectum muscular layer		11			1.0	

and gangliosides determined as earlier described (26). Individual gangliosides were isolated by chromatography on silica gel G and H (29) and identified by comparison to authentic standards.

Enzymatic determinations. Ganglioside sialidase and *p*-nitrophenylglycosidases were assayed with the methods described in a previous publication (14).

Electrophoretic separation of brain hexosaminidases in agarose gel. Plates for electrophoresis were prepared in the following way: 23 ml of a 1% agarose (Behringwerke AG Marburg/Lahn, Germany) solution in 0.04 M phosphate citrate buffer pH 6.0 heated at 60°C for 30 min was cast on a preheated 10 × 20 cm glass plate. The plate was aged for 24 hr at 4°C in a moist chamber before use. The 0.04 M phosphate citrate buffer pH 6.0 was used as electrode buffer. Contact between electrode vessels and gel was achieved by cellulose tissues 7 cm apart on the gel. Slots for 6 samples were cut in the middle of the plate.

Tissue homogenate. A 5% (w/v) homogenate of frozen samples of brain tissue was prepared by homogenizing by ten strokes in ice-cold distilled water in an all-glass homogenizer. The homogenate was then frozen and thawed three cycles in solid carbon dioxide/ethanol. 10–15 μ l of each sample was applied to the agarose plate. Electrophoresis was performed with a voltage of 30 V/cm for 30 min with cooling. Indication of hexosaminidase activity the plate was incubated for 1 hr at 37°C in 0.001 M 4-methylumbelliferyl β -D-2-acetamido-2-deoxyglucopyranoside (Koch Light Laboratories Ltd, Colnbrook, England) in 0.1 M citrate buffer pH 4.4. The substrate solution was allowed to drip off and the plate was then gently sprayed with 1 M glycine Na-carbonate buffer pH 10.0. The location of fluorescent enzyme active fractions was viewed under a Desaga UV lamp at 366 nm.

When normal cerebral cortex was investigated two enzyme fractions were distinguished. One moving anodically corresponding to the hexosaminidase fraction A described by Robinson & Surlin (18) and Okada & O'Brien (15). The other fraction B migrated slowly towards the cathode.

Ultracentrifugation of brain tissue. A 5% homogenate was prepared as for the electrophoresis experiments. The homogenate was centrifuged at 105 000 g (rotor 40 40 000 rpm) for 1 hr at 0°C in a Spinco L ultracentrifuge. The supernatant was retained and the sediment reconstituted with distilled water and homogenized.

RESULTS

Gangliosides and oligohexosides. The concentration of gangliosides was increased 4 to 8 fold in necropsy specimens of the cerebrum and cerebellum in the subjects of Tay-Sachs disease (Table 1). The increase of the ganglioside concentration was roughly equal in spinal cord and spinal roots. Ganglioside G_M constituted about 90% of the total gangliosides in the entire central nervous system.

Of the other visceral organs the adrenals, the liver and the spleen contained gangliosides in higher concentrations than the other organs examined (Table 1). The ganglioside pattern was not so uniform as in the nerve tissue but two gangliosides G_M and G_{M3} predominated. In the other visceral organs the ganglioside concentration was only 1/5–1/10

stepwise manner, each step being catalyzed by a presumed specific enzyme (for review see 4 28) with one exception the gangliosidase which does not seem to be specific for any particular ganglioside

In the inherited diseases of lipid metabolism leading to the storage of specific lipids in the tissues the lipidoses the known enzymatic defect has always been on the catabolic side. Therefore one might postulate that all inherited diseases with a primary storage of a certain ganglioside the gangliosidoses are caused by a lesion of a catabolic ganglioside enzyme. In normal human brain the first step in the degradation of the major gangliosides G_{D1} and G_{T1} involves the release of a sialic acid (9 13 28). No disease is known in which these gangliosides are accumulated which means that no inherited failure of gangliosidase activity is known. The figure for the gangliosidase activity in the cerebral cortex of one of our cases of Tay Sachs disease was comparatively low but within the normal range for age.

The monosialogangliosides G_{M1} , G_{M2} and G_{M3} (Fig 1) which are intermediates in the degradation of G_{D1} to lactosylceramide have all been shown to be stored in the respective forms of gangliosidoses. These three major forms of gangliosidoses have been named G_{M1} , G_{M2} and G_{M3} -gangliosidosis according to the type of ganglioside stored (27).

Theoretically the clinical symptoms and signs and the pathological anatomical picture should be the same irrespective of the type of ganglioside stored. Identical or very similar morphological features have been shown in the nervous system (3 7 12 24). The clinical manifestations however differ between the three forms of gangliosidoses but also within a given form.

The clinical manifestations of the only patient with G_{M3} -gangliosidosis on record (7) differed from those of classical Tay Sachs disease by massive splenomegaly. Histological examination of lymphatic tissue showed a replacement of the normal tissue elements by

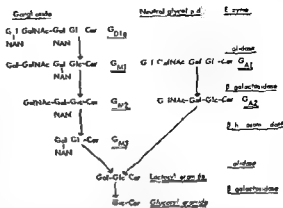


Fig 1 Degradation of gangliosides and corresponding neutral glycolipids of brain. Cer = ceramide, Gal = N acetylgalactosamine, Glc = glucose, NAN = N acetylneuraminic acid.

foam cells. The nervous system contained large deposits of ganglioside G_{M3} and lactosylceramide (17).

The onset and clinical course of the most common form of G_{M1} gangliosidosis resemble those of classical Tay Sachs disease (21) but in the former also the viscera and skeleton are involved. Because of its generalized nature this form of G_{M1} gangliosidosis has also been called "generalized gangliosidosis" (12). It was originally claimed (12) that the storage of G_{M1} ganglioside was responsible for the extraneural manifestations. The normal major brain gangliosides (G_{M1} , G_{D1} and G_{T1}) and G_{M3} occur only in minute amounts in extraneural organs. Yet a 100-fold increase of G_{M1} and G_{M2} may occur (Table 1 24) in the respective gangliosidosis. It is not large enough to produce clinical symptoms. Suzuki et al (24) later showed extensive storage of two mucopolysaccharides, keratan sulfate and a sialomucopolysaccharide. An almost complete lack of β -galactosidase activity has been demonstrated both with *p*-nitrophenyl β -galactoside and G_{M1} substrates (16). In a more uncommon variant of G_{M1} gangliosidosis (2) the onset is later, the course slower and the clinical manifestations dominated by psychomotor retardation and ataxia without any obvious extraneural symptoms. In many respects it

Table 3 Activity of ganglioside sialidase in some disorders affecting brain lipid metabolism

The assay was performed on frozen specimens of frontal brain cortex

Brain tissue	Age	Sialidase activity nmoles released NAN per mg tissue protein (M±S D)
Controls	0-21 months (n=8)	2.2±0.7
Controls	3-85 y (n=21)	6.0±1.1
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Infantile metachromatic leucodystrophy	3 y	3.4
Globoid cell leucodystrophy	1 y	5.1
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in control material. In JAI and MLD the hexosaminidase activities were increased 3-5 times.

Subfractionation of β glycosidase activities by ultracentrifugation. Homogenates of brain cortex from the 3 year old girl with Tay Sachs disease and from a control were subjected to high speed centrifugation. The sediment and supernatant fractions obtained were assayed for glycosidase activities (Table 2). In the control material, the β glucosidase activity was exclusively particle bound and was recovered in the sediment. About 2/3 of the β N acetylgalactosaminidase and β N acetylglucosaminidase activities were obtained in the supernatant. The glycosidases from cerebral tissue from the girl with Tay Sachs disease showed a similar distribution except for the two hexosaminidases both of which were found exclusively in the sediment fraction.

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Brain ganglioside sialidase. The activity of ganglioside sialidase was determined in samples of cerebral cortex from the 3 year-old girl with Tay Sachs disease, from GCLD, MLD, JAI and MS (Table 3).

The sialidase activity in the TS patient was low and that in the GCLD patient high compared to age matched controls while in the other diseases studied the activities were within the range found for the controls. A search was made for the possible presence of inhibitory factors affecting the assay of ganglioside sialidase. Mixtures of separately assayed enzymes prepared from normal brain tissue and from pathological material were incubated. The following recoveries of enzyme activity was observed: Tay Sachs disease 106%, MLD 97%, JAI 98% and GCLD 95%.

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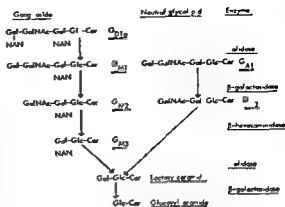


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DISCUSSION

The biosynthesis and biodegradation of brain gangliosides have been shown to occur in a

advantage that the material can be used for microscopical examination

Previous methods for subfractionation of the hexosaminidases (6 15 19) were too complicated and time-consuming for routine determinations. In the agarose gel electrophoresis method elaborated in the present study the same equipment and technique were used as for routine serum protein electrophoresis. The enzymatic determination can be performed on material from many sources but liver or white blood cells seem to be the most convenient. After this study had been concluded an acrylamide gel electrophoretic method was described which has been adopted for the identification of Tay Sachs disease carriers (5). A fluorimetric method has also been elaborated for the assay of hexosaminidase A in serum which may be useful for the detection of homozygotes and heterozygotes for Tay Sachs disease (11).

Tay Sachs disease was originally classified among the amaurotic idiocies but it is now generally accepted to designate a gangliosidosis after the chemical structure of the stored ganglioside as first proposed by Suzuki & Chen (23). A subdivision of the gangliosidoses according to the enzymatic abnormality was recently suggested by one of us (27) and by O'Brien (10) variant forms being described as types I II etc. A more sophisticated nomenclature for the enzymatic subdivision of the G_M -gangliosidosis has also appeared (31). We consider that a more comprehensive system of enzyme nomenclature should not be used until specific ganglioside substrates have been applied and the relation between the two hexosaminidases and G_M and neutral aminoglycolipids has been established.

Young et al. (31) have also discussed the use of the clinical designation Tay Sachs disease. Volk (30) has used the term synonymous with amaurotic idiocy and thus included diseases with no disturbances of ganglioside metabolism (27). The other extreme would be to use the name only for the infantile form of G_M -gangliosidosis variant II in which hex-

osaminidase A is missing and hexosaminidase B is increased (Table 4). Since the three forms of infantile G_M -gangliosidosis apparently produce the same clinical manifestations we consider it justified to use the name "Tay Sachs disease" synonymously with infantile G_M -gangliosidosis.

SUMMARY

Gangliosides and neutral aminoglycolipids were determined in several organs from two subjects with classical Tay Sachs disease. Ganglioside G_M was stored in all organs investigated. Of the extraneural organs the highest concentration was found in the adrenals, spleen and liver. The rectal muscular layer had a high concentration of gangliosides. Rectal biopsy might be used for studies of the nervous ganglioside pattern.

The brain tissue showed a many fold increase of the activities of *p*-nitrophenylglycosidases. The activity of ganglioside sialidase in the brain was not increased in Tay Sachs and some other neurometabolic diseases. Subfractionation of the glycohydrolases by ultracentrifugation showed no hexosaminidase activity in the supernatant fraction of the brain in Tay Sachs disease compared with about 2/3 of the total activity in normal human brain.

A simple agarose electrophoretic method was developed for the separation of the hexosaminidases into two fractions A and B. Hexosaminidase A was virtually absent in Tay Sachs disease.

The known forms of gangliosidoses and their biochemical characteristics are reviewed and a scheme is given for their classification and diagnosis.

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Table 4 The classification of the gangliosidoses

Suggested name	Trivial name	Enzyme abnormality
<i>G_{M1}</i> Gangliosidosis		
Infantile form	Norman-Landing disease	<i>G_{M1}</i> and <i>p</i> -nitrophenyl β galactosidase <10% of normal activity <i>p</i> -nitrophenyl β galactosidase low
Late infantile form	Generalized gangliosidosis	
<i>G_{M2}</i> -gangliosidosis		
Infantile form		
Variant I	Tay-Sachs disease	Hexosaminidase A absent
Variant II	Tay-Sachs disease	Hexosaminidase B absent
	(classical form)	Hexosaminidase A absent
Variant III	Tay-Sachs disease	Hexosaminidase II high
		Hexosaminidase A high
		Hexosaminidase B high
Late infantile form		Hexosaminidase A low
Juvenile form		Hexosaminidase B normal
<i>G_{M3}</i> gangliosidosis		
Infantile form	Neurovisceral gangliosidosis	

resembles the late infantile form of amaurotic idiocy described by Jansky & Bielchovsky (22)

The biochemical characteristics of patients with classical Tay Sachs disease are the large accumulation of *G_{M2}* ganglioside in nervous tissue, a many fold increase of *G_{M2}* in extra neural organs, a moderate increase of neutral aminoglycolipid *G_A* in the brain and little or no increase of globoside in visceral organs. The activity of β hexosaminidase in tissues and serum is increased (11-15) but fraction A is missing.

Variant forms have recently been described (Table 4). Jatzkewitz and collaborators (20) have described one patient with a clinical record of classical Tay Sachs disease but with transient enlargement of the spleen and liver. In that patient the brain content of *G_{M2}* was the same but *G_A* was much larger than in the present two cases of Tay Sachs disease. An extensive storage of globoside was found in the kidney and histochemical studies revealed signs of glycolipid storage in the spleen and liver. The hexosaminidase activity assayed both with the *p* nitrophenyl *N* acetyl β glucosaminide and -galactosaminide and labeled globoside and *G_A* was only a few percent of normal. In a systematic study of hexosaminidases from brains of subjects with

a history of all the typical symptoms and signs of Tay-Sachs disease Sandhoff (19) found increased activities of both hexosaminidases A and B in one case. Also, a late infantile and a juvenile form of *G_{M2}* gangliosidosis have been reported. In the late infantile form the clinical manifestations were the same as those described by Jansky and Bielchovsky for late infantile amaurotic idiocy (8). The content of *G_{M2}* ganglioside was almost as high in the brain as in Tay-Sachs disease, and *G_A* was also shown in the liver. Hexosaminidase A activity was diminished (31).

The biochemical diagnosis of a gangliosidosis should consequently include the identification of the stored ganglioside and the determination of the enzymatic abnormality. The investigation can be performed early in the course of the disease by examination of a biopsy specimen of the brain. The present study has shown that also some other organs can be used for identification of the stored ganglioside. The second largest relative accumulation of *G_{M2}* occurred in the liver but the separation of *G_{M2}* from the normal *G_{M3}* may be difficult. The muscular layer of the rectum has a nerve ganglioside pattern and the chromatographic characterization of the gangliosides is simpler. Rectal biopsy has also the

A CONTROLLED TRIAL ON THERAPY FOR NEWBORNS WEIGHING 750-1 250 g

I Clinical Findings and Mortality in the Newborn Period

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Hitherto few investigations have been devoted solely or extensively to therapeutic procedures capable of decreasing the high mortality rate in newborns with very low weight (14 43 44). The intravenous infusion with glucose and sodium bicarbonate has been shown to improve survival in controlled trials on newborns with respiratory distress syndrome (RDS) weighing more than 1 25-1 50 kg (37 43). This procedure has been advocated routinely also for smaller newborns with or without RDS (3). However the experimental evidence on the benefit of intravenous infusion as compared with oral feeding in very small newborns is still limited and inconclusive. In fact in one trial (14) there was some suggestion but no conclusive proof of a decrease in mortality after intravenous infusion whereas no change in neonatal survival was found in another study (43).

Although the reported incidence may vary from one institution to the other infection and hemorrhage are common causes of death in newborns with very low weight (12). This was confirmed in a preliminary investigation in this Centre (31) on a consecutive series of 27 autopsies in newborns weighing 750-1 250 g in this series, the incidence of pneumonia was 26% and the incidence of intracranial hemorrhage 52%. Therefore the problem of an ef-

fective antimicrobial therapy (18) and of the correction of possible coagulation defects (17) needs to be considered.

We were thus led to perform a controlled therapeutic trial in newborns with birthweight of 750-1 250 g. Two therapeutic regimens were contrasted: one of very active therapy with intravenous infusion with glucose and sodium bicarbonate 3 antibiotics gamma globulins and fresh human plasma (the "treatment series"), the other with standard oral feeding and a single broad spectrum antibiotic (the control series). In the present paper, clinical findings, mortality data and post mortem observations in the neonatal period will be presented. Laboratory and cardiac observations have been given in another report (11). Follow-up studies are also in progress.

MATERIAL AND METHODS

All subjects who met the following requirements entered the trial: 1) weight on admission of 750-1 250 g; 2) admission, as well as acid base and the X ray studies, within 24 hours of birth; 3) no major congenital malformation or hemolytic disease on first clinical and laboratory examination. Assignment to the treatment or the control group was performed as follows. Infants were stratified in four subgroups according to their weight and blood pH on admission (weight above/equal or below 1 090 g, and blood pH above/equal or below 7.27—these were the median weight and pH values in a consecutive series of

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Table 1 Plan of therapy in the treatment and control groups

Treatment group	Control group
Fluid and calories I v infusion 70 ml/kg/day (10 glucose + NaHCO ₃) for at least 4 days Oral feeding (only pooled human milk) starting in 2nd day of life stepwise increase up to 150 ml/kg/day by 7th-8th day	Oral feeding none on 1st day 10 glucose (25 ml/kg/day) from 2nd to 5th day pooled human milk 25 50 75 125 150 175 ml/kg/day from 3rd to 8th day
Antibiotics Sodium penicillin G 500 000 U + methicillin 100 mg + colistin 8 mg per kg/day for 5-7 days (i v i m after i v stopped)	Kanamycin sulf (40 mg/kg/day i m) in first 5 days Sodium penicillin (100 000 U i m) after 5th day when required
Other therapies Gammaglobulin preparation 0.5 ml/day in first 3 days fresh human plasma 3 ml every 2nd day in first 5 days Vitamin K ₁ (3 mg i m) on admission oxygen when needed (up to 100 conc) ambient temperature 32-34 C or servocontrol of body temperature	

infants at 2-6 hours in 13 at 7-12 hours in 9 at 13-18 hours and in 3 at 19-24 hours) and it was discontinued between the 4th and the 7th day. Oral feeding was started between 22 and 50 hours of birth (with the only exception of 5 infants who were fed at 51-60 hours). In the controls the first feeding was given on the average at 24.5 hours of age (in 2 subjects between 29 and 39 hours and in the remaining subjects between 20 and 28 hours).

The mean amount of fluid and calories given per day to treated and control babies in the neonatal period has been reported on Table 4.

The most marked contrast was present in the first 3 days of life when treated infants received almost three times as much fluid and calories than the controls. The difference was small in the 4th-5th day and disappeared by the 6th-8th day of birth.

Outcome and post mortem findings

The mortality rate in the first 10 days of life in the 80 infants studied was 61.2%. Data on mortality and the most relevant post mortem findings in the experimental groups have been summarized in Table 5. The neonatal survival curve has been illustrated in Fig. 1. None of

Table 2 Some characteristics of infants in the trial

	Treated	Controls
Number of cases	40	40
No. (n) & birthweight < 1 kg	18 (45%)	17 (42%)
No. (n) & gest age < 38 weeks	2 (6%) ^a	19 (61%)
No. (n) & weight < 10th perc for gest age ^a	5 (16%) ^b	3 (10%)
No. (n) males	17 (42%)	21 (52%)

^a According to the single birth intrauterine growth chart of Lubchenko et al. (27).

^b Known in 32 infants, known in 31 infants.

Table 3 Clinical and laboratory findings (mean \pm S.D. or %) on admission in infants in the trial

	Treated	Controls
Number of cases	40	40
Age hours	6.8 \pm 5.5	6.5 \pm 4.9
Silverman's score	3.8 \pm 2.4	3.6 \pm 1.8
Respirat. rate min	51.1 \pm 10.3	55.0 \pm 11.0
No. (n) & marked X-ray abnormality	13 (32%)	10 (25%)
SBP mmHg	39.5 \pm 5.9	40.9 \pm 8.5
Arterial acid-base status		
age of study hours	8.5 \pm 5.6	8.8 \pm 5.2
pH	7.311 \pm 0.140	7.329 \pm 0.090
Base Excess mEq/l	-7.2 \pm 5.5	-7.2 \pm 4.0
Pco ₂ mmHg	44.2 \pm 17.9	37.7 \pm 14.2

40 babies with the same weight range, previously admitted to the unit) Within each subgroup subjects were included in the treatment or in the control series by means of sealed envelopes. It was established in advance that the trial was to be stopped after studying 40 treated and 40 control infants.

The plan of treatment has been summarized on Table 1. Additional explanations are as follows. The aim of NaHCO_3 administration was to keep the blood Base Excess as near as possible to 0 mEq/l or at most slightly above such level in order to compensate for hypercarbia (but no attempt was made to completely compensate for severe hypercarbia). For this purpose an initial dose of NaHCO_3 (mEq given = blood Base Deficit mEq/l \times body weight, kg \times 0.5) was given in 2-6 hours and additional amounts were administered subsequently when required with monitoring of the arterial acid base status. The infusion was given continuously at the rate of 70 ml/kg/day by a No. 5 French polyvinyl catheter in the umbilical vein; it was maintained for at least 4 days and it was stopped only when no major therapeutic problems were considered to be present. Heparinized plasma was given within 2 hours of collection from the donor. Supplemental oxygen was administered to patients with cyanosis, respiratory distress or apnoeic spells, and according to arterial oxygen studies when available. Apnoeic episodes were treated only by bag and mask ventilation. In the first part of the investigation only incubators with manual regulation of ambient temperature were used. In the last part of the trial incubators with servo-regulation of temperature became available providing 12 treated and 11 control subjects with a skin temperature of 36-36.5°C. Patients with bradycardia associated with 2:1 atrio-ventricular block (9) were treated with 1% CaCl_2 intravenously.

The gestational age was calculated from the first day of the last menstrual period and rounded to the nearest week. Clinical observations included among others repeated recording of Silverman's score, respiratory and heart rate and skin colour. In many patients a detailed neurological examination was repeatedly performed by one of us (S.P.B.).

The systemic systolic blood pressure (SBP) was measured indirectly from the radial artery by a modified xylol/pulse indicator instrument (7). The difference was also calculated between observed value and normal average value predicted from body weight, gestational age and postnatal age ($\Delta \text{SBP} = \text{mm Hg} - \text{observed} - \text{predicted SBP}$) according to a multiple regression equation obtained in another study (10).

Antero-posterior chest films were taken in duplicate on admission by a portable X-ray machine (focal distance 90 cm, exposure time 0.04-0.06 sec, inten-

sity 50-55 kV) without removing the infant from the incubator and were repeated when required. Retrospectively, the chest X-ray findings were classified as: 1) markedly abnormal (i.e. with severe and diffuse hypotransparency and/or fine granularity, coarseness, nodularity, large patchy opacity); 2) without marked abnormality (i.e. absence of findings listed under 1).

Acid-base and oxygen determinations were performed on arterial (radial) or arterialized capillary blood by means of a micro-Astrup apparatus and a Clark-type microelectrode. Carbon dioxide tension (Pco_2), oxygen tension (Po_2) and pH were corrected for the temperature of the infant. Additional details on methods of sampling, measurement and calculation have been described elsewhere (8, 3). Other laboratory determinations and ECG tracings were also obtained, and have been reported elsewhere (11).

After microscopic examination of the lungs, the findings of interstitial and/or alveolar polymorphonuclear infiltration and of hemorrhage were graded from 0 to 3.

Differences in average values were analysed by the Student's *t* test. Differences in the mortality rate or in the prevalence of various situations were analysed by the χ^2 method. Differences in survival curves were analysed by the Wilcoxon two sample test (rank-sum test) assigning ranks to the survival time in dead infants and assuming a normal distribution for survivors (15).

RESULTS

The trial was performed from December 1965 to July 1968.

During this period 17 babies admitted within 4 hours of birth and weighing 750-1250 g were not included for the following reasons: died immediately after admission before completion of laboratory studies (5); acid-base studies not available because of failure of laboratory equipment (7); congenital malformation (2); stratified subgroups (see previous paragraph) already completed (3). 12 out of the 17 patients (71%) died within the first 10 days of birth.

Findings on admission, and comparability of experimental groups

Some characteristics of treated and control babies have been reported in Table 2 and some clinical and laboratory findings in Table 3. The differences between the experimental groups were small and statistically not significant.

Fluid and feeding actually administered

In treated babies the infusion was started on the average at 10 hours of age (in 15 m-

* Regression equation for SBP of healthy low weight newborns (age 3-96 hours)
 $\text{SBP (mm Hg)} = 23.2 + 8.13 \text{ bw} + 0.503 \text{ ga} + 0.226 \text{ pna} - 0.0016 (\text{pna})^2$
 where bw = body weight (kg), ga = gestational age (weeks) and pna = postnatal age (hours)

Table 6 Neonatal mortality in the whole series and in treated or control subjects according to some findings on admission

	All subjects			Treated		Controls		p treated vs. contr
	No of cases	% died	p	No of cases	% died	No of cases	% died	
Birthweight, kg								
>1.04	40	45%	}	21	48%	19	42%	}
<1.04	40	77%		19	84%	21	71%	
Gest. age weeks								
>48	35	37%	}	19	53%	16	19%	}
<48	28	75%		13	77%	15	73%	
Sex								
males	38	63%		17	65%	21	62%	
females	42	60%		23	65%	19	53%	
Resp. rate/min								
<54	41	76%	}	22	68%	19	84%	}
>54	39	45%		18	61%	21	33%	
Chest X ray findings								
no marked abnormal	57	54%		27	56%	30	47%	
marked abnormal	23	76%		13	85%	10	70%	
Arterial pHi								
>7.323	39	44%	}	15	47%	24	42%	}
<7.323	41	78%		25	76%	16	81%	

Significance of difference in mortality rate * $p < 0.05$ ** $p < 0.02$ *** $p < 0.01$ *Apnoeic spells and neurological examination*

Comparison was made between the number of treated and of control infants with apnoeic spells in the first 3 days and from 4 to 10 days of birth (separated also into those with 1 or 2 and those with more spells). Spells occurring within 2 hours of death were not considered. No significant differences were found.

In some of the infants a thorough neurological examination was performed at random by one observer (S. P. B.). Out of the various findings elicited only the occurrence of tremors and clonus and the pattern of muscular tonus in the 2nd-3rd day of life were selected for presentation. (When different findings were observed in the 2nd and in the 3rd day only observations in the 2nd day were considered.) 23 treated and 26 controls were studied. The incidence of tremors was very similar in the two groups. The incidence of clonus and of increased muscular tonus was slightly higher in controls (clonus 9% in treated and 19% in control subjects; increased tonus never in treated and 15% in controls) but the difference was not statistically significant. Seizures were never observed.

Heart rate and systolic blood pressure

Persistent bradycardia (i.e. heart rate below 85/min) not associated with apnoea or with agonal conditions was found in the 2nd day of life in 6 control patients. This was always associated with 2:1 atrio-ventricular block, hyperkalemia and hypocalcemia and was cured by variable amounts of 1% CaCl₂ intravenously. These patients have been more extensively considered in another report (11).

In the 63 subjects with known gestational age 198 SBP determinations were performed in the first 4 days of life and the Δ SBP values were calculated. The mean Δ SBP in treated and control babies at various age intervals (1st, 2nd, 3rd and 4th day of birth) was compared, and no significant difference was found.

Fresh human plasma administration and hemorrhage at post mortem examination

According to the plan of the trial (Table 1) 3 ml/kg of fresh human plasma were to be administered to infants in the treatment group as soon as possible after admission and to be repeated in the 3rd-4th day and possibly also

Table 4 Mean amount of fluid and calories (oral + i.v.) actually administered to treated and control babies in the first 8 days of life (Amounts given in the day of death have not been considered)

Day of life	Fluid ml		Calories	
	Treated	Controls	Treated	Controls
1	41	1	17	0.4
2	112	223	28	80
3	100	51	38	98
4	119	90	43	14
5	138	257	63	26
6	139	119	78	46
7	145	130	87	71
8	162	145	97	85
		171	108	297
				115

the reported differences was statistically significant. The most marked (but not significant $p=0.07$) discrepancy was represented by a higher incidence of intracranial hemorrhage in treated infants (48%) than in controls (23%). No central thrombosis or hepatic lesions were observed on gross post mortem examination. However a very detailed examination of abdominal vessels together with microscopic studies of the liver had not been planned in advance and were rarely performed.

Table 5 Outcome and post mortem findings in the experimental groups

	Treated	Controls
Number of cases	40	40
No () died	26 (65%)	23 (57%)
Median age of death, hours	81	42
No of autopsies	25	22
No () = intracranial hemorrhage	12 (48%)	5 (23%)
No () = visceral ^a hemorrhage	0	1 (5%) ^d
No () = other findings	0	2 (9%) ^e
No of lung microscopic examinations	21	21
No () = HMD	5 (24%)	6 (29%)
No () = pneumonia ^b	2 (9%)	1 (5%)
No () = hemorrhage ^c	4 (19%)	4 (19%)

^a Pulmonary hemorrhage not included

^b Grade 2 & 3 polymorphonuclear infiltration

^c Grade 3 interstitial and/or alveolar hemorrhage

^d Intestinal

^e 1 = left kidney & ovary agenesis and 1 = moderate ileal stenosis

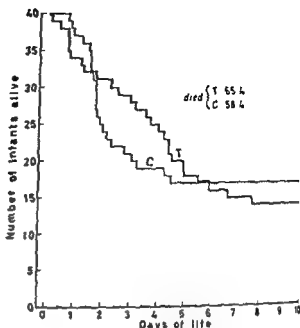


Fig 1 Neonatal survival curve in treated (T) and control (C) infants in the trial

Prognostic value of some findings on admission, and therapy

The possible effect of treatment on the mortality rate was also investigated after comparing the infants according to some findings on admission. The latter included above or below median birthweight, gestational age, respiratory rate and arterial pH, sex, absence or presence of marked abnormality on chest film.

In Table 6 the mortality rate in such categories of subjects has been reported. When considering all infants (irrespective of treatment), a significantly lower mortality was found in subjects with higher birthweight, gestational age, respiratory rate and arterial pH, whereas no significant differences were observed between males and females, or between infants with or without marked chest film abnormality. When comparing treated and control infants within the same category the only significant difference ($p < 0.05$) was found in the category with gestational age > 28 weeks, with a lower mortality rate in control subjects. However, in the overall context of the present results it is difficult to assign a definite meaning to this observation.

compared to controls needs some comment. It has been suggested that intracranial hemorrhage may result from osmolar load (25). However in the present study the difference in the incidence of intracranial hemorrhage between the experimental groups did not attain statistical significance. In addition, in a controlled trial on babies with HMD and birthweight of 1 250-2 500 g performed in this Centre (37) a similar incidence of intracranial hemorrhage was found in babies with oral feeding or with i.v. infusion of glucose and NaHCO_3 . Thus the conclusion seems warranted that none of the therapeutic regimens tested influenced *per se* neonatal mortality.

The role of intravenous infusion with glucose and alkali in the therapy of newborns with very low weight

In newborns with the respiratory distress syndrome a decrease in mortality rate after alkali and glucose infusion has been observed by many workers (21-23, 40). However Usher (43) reported improved survival in patients with birthweight above 1.5 kg, not in those with birthweight of 1-1.5 kg. In this Centre a controlled trial was performed on babies weighing 1 250-2 500 g and an improved neonatal survival curve was found after infusion (37).

In newborns with birthweight of 750-1 500 g (with and without respiratory disease) Cornblath et al. (14) found no significant mortality changes after i.v. infusion with glucose (and without NaHCO_3) as compared to oral feeding; however when analysing retrospectively the results in the group with birthweight of 750-1 250 g, a significant decrease in mortality after i.v. infusion was found.

The present trial has provided conclusive evidence that, under our experimental conditions, no detectable improvement of neonatal survival could be obtained by i.v. infusion therapy in subjects with very low birthweight and with or without pulmonary disease, or early acidosis. Similar results were obtained in a controlled trial previously performed in this Centre on 40 infants with the same weight

(31). In that trial the plan of oral or i.v. fluid administration was the same as in the present investigation with the exception that infants in the treatment group were given the infusion only if the arterial pH was below 7.30 in the early days of birth.

However in previous studies on newborns with low weight hypoglycemia, metabolic acidosis, increased levels of NPN, K and P and increased urinary output of Na, K, and N were frequently observed; these changes were enhanced by fasting, hypothermia and respiratory distress and were prevented or corrected at least in part by early oral feeding or infusion therapy (2, 4, 5, 8, 13, 16, 19, 24, 28-30, 32, 34, 35, 38, 39, 41, 42, 45, 46). In some investigations prevention of hyperbilirubinemia by early fluid administration has been reported (20, 26, 39, 45, 46). Some of these findings have been substantiated by laboratory observations performed in the course of the present trial (11). Since many of these abnormalities are potentially dangerous to the central nervous system, the final evaluation of the role of intravenous infusion with glucose and alkali in newborns with very low weight must await long term follow up studies in survivors. Thus it is suggested that, before such studies are available, this therapy should be given routinely to newborns with very low weight. The problem of duration of the infusion has been discussed elsewhere in greater detail (11).

Antimicrobial therapy, mortality and pneumonia

In previous studies there was some suggestion but no conclusive proof that the administration of methicillin plus colistin (36) or of gamma globulin (1) was beneficial to low weight newborns. In the present trial there was no difference in mortality rate after the two antimicrobial regimens tested (Tabl. 1) and the incidence of pneumonia at post mortem examination was low both in the treated (9%) and in the control (5%) series. Bacteriological studies were not performed routinely and therefore could not be used in order to evaluate

Table 7 Incidence of significant hemorrhage upon post mortem examination in patients with or without plasma therapy

	Autopsy			Lung microscopic examination	
	Total no	No () δ intracranial hemorrhage	No () δ other hemorrhage ^a	Total no	No () δ hemorrhage ^c
Subjects with plasma therapy	20	10 (50 %)	0 (0 %)	17	3 (18 %)
Subjects without plasma therapy	27	7 (26 %)	1 (4 %) ^b	25	5 (20 %)

^a Not including pulmonary h^b Intestinal^c Grade 3 pulm hemorrhage

in the 5th-6th day of life. However, because of practical difficulties, this plan was not followed in every case. In fact, 6 infants (4 living less than 24 hours, and 2 living for 2 days) never received plasma. 17 babies (7 living less than 72 hours, 6 living for 4-5 days, and 4 surviving) received only 1 administration. Only 17 subjects received 2 or 3 administrations.

Thus, the 47 patients in this series with post mortem studies were divided into two groups: the 20 infants in the treatment group who did actually receive at least 1 plasma administration and the 27 infants (including 5 babies in the treatment group) who never received plasma. Post-mortem studies have been reported in Table 7. The incidence of intracranial hemorrhage was markedly but not significantly higher in subjects who received plasma (50%) as compared with those without (26%) plasma therapy. The incidence of hemorrhage in other organs was fairly similar in the two groups.

Major observations after the neonatal period

Retrolental fibroplasia was never observed on the routine fundoscopic examination performed before discharge. Two infants belonging to the control group developed the pulmonary syndrome of Wilson Mikity and died at 2-3 months of age. The remaining 29 babies were discharged from the Unit in the 3rd-4th month of life in apparently good health.

DISCUSSION

Methodology

In the present trial a satisfactory comparability of the experimental groups was achieved by stratifying subjects on enrollment according to two prognostic parameters (i.e. weight and arterial pH). Luckily enough, these parameters proved retrospectively to be among the best discriminants with respect to neonatal mortality as compared with other prognostic factors considered in Table 6. The need for a careful selection of parameters when stratifying for experimental groups in controlled trials is apparent.

The treatment schedule differed from the control regimen by three respects (oral feeding vs. i.v. infusion, antimicrobial regimen, fresh human plasma administration). This could have made the interpretation of results difficult if a difference in neonatal survival had been found between experimental groups. However, no such difference was observed. In addition, there was no evidence of beneficial effects of either antimicrobial regimens tested or of plasma administration when considering post mortem findings. Nor was there any evidence of possible adverse effects of either antimicrobial regimens, umbilical vein catheterization or intravenous therapy. With respect to the latter statement, the finding of a markedly higher incidence of intracranial hemorrhage at post mortem examination in treated babies as

vented or corrected at least in part, by the early infusion with glucose and NaHCO_3 . Since some of these abnormalities are potentially dangerous to the central nervous system the final evaluation of this therapy must await long-term follow up studies in survivors. It is therefore suggested that before such studies are available the early i.v. infusion with glucose and NaHCO_3 should be given routinely in newborns with very low weight.

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the effects of treatment. The present evidence, although not conclusive, suggests that in our experimental conditions no difference in mortality due to pneumonia can be expected after either therapy.

It may be of some interest to report that the present antimicrobial trial was performed because in a previous series (31) of autopsies in newborns with similar weight given kanamycin sulf (20 mg/kg/day for 5 days), the incidence of pneumonia was 26%. This observation clearly shows that the population admitted to a given unit may change considerably in character with time, and that policies of antibiotics administration need to be continuously reevaluated. The authors fully agree that antibiotics should be administered to low-weight newborns only when infection is definitely suspected. However, they feel that in their working conditions routine antibiotic administration for a few days was justified, since often the previous history of their referred patients was unreliable or long in gathering.

Fresh human plasma administration, mortality, and hemorrhage

In the present investigation mortality and the incidence of hemorrhage at post mortem examination were similar in infants who did or did not receive fresh human plasma (3 ml/kg in the first day of life, sometimes repeated in the 3rd and 5th day). On the other hand Gray *et al* (17) reported that the administration of 10 ml/kg of fresh human plasma to low weight newborns with clotting defects (as assessed by Thrombotest) lessened significantly the risk of death with intracranial hemorrhage. Further investigation is clearly necessary including the serial evaluation of single coagulation factors before and after therapy.

SUMMARY

A controlled therapeutic trial was performed in newborns with birthweight of 750–1 250 g. 40 infants (the treatment group) received an i.v. infusion with glucose and NaHCO_3 from

the 1st to the 4th–7th day of life, and increasing amounts of human milk from the 2nd day of life. They also received, during the newborn period, 3 antibiotics (sodium penicillin G 500 000 U + methicillin 100 mg + colistin sulf. 8 mg per kg/day), a gamma globulin preparation (0.5 ml/day for 3 days) and fresh human plasma (3 ml every 2nd day in the first 5 days of birth). 40 infants (the control group) received only oral feeding with 10% glucose and human milk, starting in the 2nd day of life, and 1 m kanamycin (20 mg/kg/day for 5 days).

No difference between treated and control babies was observed with respect to the following findings: neonatal mortality rate and survival curve on the whole series, neonatal mortality rate in babies with birthweight above or below 1 04 kg, with gestational age below 28 weeks, with above or below median respiratory rate or arterial pH on admission, with or without marked abnormality on chest film and in males or females (but, in subjects with gestational age above 27 weeks, a significantly lower mortality rate was found in controls), post mortem findings, incidence of apnoeic spells, tremors and cloni, and pattern of muscular tonus. Bradycardia associated with 2:1 atrioventricular block was observed in 6 controls in the 2nd day of birth. No adverse effects, presumably due to the therapeutic procedures, so contrasted were demonstrated.

It was concluded that in the present series of newborns with very low weight (a) the massive anti-infectious therapy was not superior to the administration of a single broad-spectrum antibiotic, (b) the administration of fresh human plasma was not effective in preventing hemorrhage (but further evaluation is needed), (c) the routine i.v. infusion with glucose and NaHCO_3 did not improve neonatal survival as compared with oral feeding.

However, previous studies, as well as observations on the present series reported elsewhere (11) have shown that several biochemical abnormalities commonly seen in orally fed newborns with very low weight can be pre-

the effects of treatment. The present evidence, although not conclusive, suggests that in our experimental conditions no difference in mortality due to pneumonia can be expected after either therapy.

It may be of some interest to report that the present antimicrobial trial was performed because in a previous series (31) of autopsies in newborns with similar weight given kanamycin sulf (20 mg/kg/day for 5 days) the incidence of pneumonia was 26%. This observation clearly shows that the population admitted to a given unit may change considerably in character with time, and that policies of antibiotics administration need to be continuously reevaluated. The authors fully agree that antibiotics should be administered to low-weight newborns only when infection is definitely suspected. However, they feel that in their working conditions routine antibiotic administration for a few days was justified, since often the previous history of their referred patients was unreliable or long in gathering.

Fresh human plasma administration, mortality and hemorrhage

In the present investigation mortality and the incidence of hemorrhage at post mortem examination were similar in infants who did or did not receive fresh human plasma (3 ml/kg in the first day of life, sometimes repeated in the 3rd and 5th day). On the other hand Gray et al (17) reported that the administration of 10 ml/kg of fresh human plasma to low weight newborns with clotting defects (as assessed by Thrombotest) lessened significantly the risk of death with intracranial hemorrhage. Further investigation is clearly necessary including the serial evaluation of single coagulation factors before and after therapy.

SUMMARY

A controlled therapeutic trial was performed in newborns with birthweight of 750–1250 g. 40 infants (the 'treatment' group) received an i.v. infusion with glucose and NaHCO_3 from

the 1st to the 4th–7th day of life, and increasing amounts of human milk from the 2nd day of life. They also received, during the newborn period, 3 antibiotics (sodium penicillin G 500 000 U + methicillin 100 mg + colistin sulf. 8 mg per kg/day), a gamma globulin preparation (0.5 ml/day for 3 days) and fresh human plasma (3 ml every 2nd day in the first 5 days of birth). 40 infants (the control group) received only oral feeding with 10% glucose and human milk, starting in the 2nd day of life and 1 m kanamycin (20 mg/kg/day for 5 days).

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However, previous studies, as well as observations on the present series reported elsewhere (11), have shown that several biochemical abnormalities commonly seen in orally fed newborns with very low weight can be pre-

vented or corrected at least in part by the early infusion with glucose and NaHCO_3 . Since some of these abnormalities are potentially dangerous to the central nervous system the final evaluation of this therapy must await long term follow up studies in survivors. It is therefore suggested that before such studies are available the early i.v. infusion with glucose and NaHCO_3 should be given routinely to newborns with very low weight.

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A CONTROLLED TRIAL ON THERAPY FOR NEWBORNS WEIGHING 750-1250 g

II Blood Chemistry and Electrocardiographic Observations in the Newborn Period

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In fasting newborns with low weight hypoglycemia (3 38 43) metabolic acidosis (6 21) increased serum levels of NPN K and P increased urinary output of Na K and N (1 3 10 29 35 48 49) and hyperbilirubinemia (26) have been frequently observed. These abnormalities were reportedly prevented or corrected at least in part by the early administration of oral feeding or of intravenous infusion with glucose and with or without NaHCO_3 (1 17 19 24 28 39 45 48 49 52 56). Early hypocalcemia was a common finding (5 14 47) and as suggested by a recent study it was possibly enhanced by the administration of NaHCO_3 (47).

In fasting newborns with very low weight metabolic acidosis (6) and signs of increased tissue catabolism (1 35 48 49) were even more frequent and severe and were associated with electrocardiographic abnormalities (7 31 33 48). However there are only limited prospective studies on blood chemistry and ECG abnormalities comparing oral feeding with intravenous infusion in large and well controlled groups of very small newborns. As to the effect on neonatal mortality published results were inconclusive. In fact, in one trial there was some suggestion but no conclusive proof of a decrease in mortality after intravenous in-

fusion (11) whereas no changes in neonatal survival were reported in another study (50). In spite of these uncertainties routine early intravenous infusion has been advocated for all newborns with very low weight (2) and is currently applied in many Centres.

Thus we performed a prospective trial on the effect of intravenous infusion with glucose and NaHCO_3 as compared with oral feeding in newborns weighing 750-1250 g. For reasons given elsewhere (32) two different antimicrobial regimens and the administration of fresh human plasma were also evaluated. In this paper blood chemistry and electrocardiographic observations in the neonatal period will be presented. Clinical findings and mortality data in the newborn period have been given in another report (32). Follow up studies are also in progress.

MATERIAL AND METHODS

The material was the same as described in the previous report (32). The plan of treatment (summarized in Table 1 in the previous communication (32)) calculation of gestational age and classification of X-ray findings were the same as reported previously (32).

Acid-base and oxygen determinations were performed on arterial (radial) or arterialized capillary blood by means of a micro Astrup apparatus and a Clark type micro-electrode. Carbon dioxide tension

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infants (for technical difficulties some electrolytes were not studied in part of the cases) Patients were selected at random but very sick subjects were usually not studied The results of blood studies some findings on admission and some data on therapy have been reported on Table 2 Upon admission treated patients had marked abnormality on chest film more seldom than the controls but their mean blood pH was lower

In the 2nd day of life the mean serum Na and Ca were identical in the two groups where as the mean serum K and P and the BUN were significantly ($p < 0.01$) higher in the controls Individual data have been illustrated in Fig. 4 from which it appears among other findings that serum K exceeded 3 mEq/l in 13 out of 22 controls (59%) and only in 3 out of 18 treated patients (16.7%) The arterial Ht did not change appreciably from 1st to

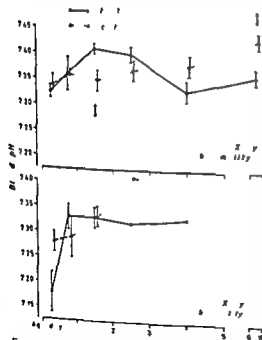


Fig. 1 Mean \pm SE of arterial pH in treated and control infants with or without severe abnormality on chest film on admission. Mean values from less than 3 observations, and SE from less than 4 observations, were not included. Arrows indicate significant differences between means $-p < 0.05$ $-p < 0.01$

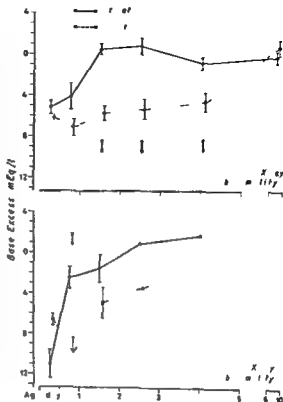


Fig. 2 Mean \pm SE of blood Base Excess in treated and control infants. Other explanations as in Fig. 1

2nd day of life in the controls and decreased moderately in treated babies

The correlation between various blood chemistry findings was investigated after pooling results in the treated and the control group. Highly significant correlations were found between serum K, serum P and BUN (Fig. 5 a, b and c). There was a weak but still significant correlation between blood pH in the 2nd day of life on the one hand and serum K or serum P on the other whereas no correlation at all could be demonstrated between blood pH in the 2nd day and BUN (Fig. 6 a, b and c). On the contrary the blood pH on admission did not bear any correlation with either serum K, serum P or BUN. The relationships between the various blood chemistry findings were apparently similar in treated and control infants in fact whenever a significant relationship was found and the regression line

Table 1 Some characteristics and some laboratory findings on admission (mean \pm SD or %) in infants in the trial

	Treated	Controls
Number of cases	40	40
No () & birthweight <1 kg	18 (45 %)	17 (42 %)
No () & gest age <28 weeks	22 (66 %) ^a	19 (61 %) ^c
No () & weight <10th perc for gest age ^a	5 (16 %) ^b	3 (10 %) ^c
Age on admission hours	68 \pm 5.5	65 \pm 4.9
No () & marked abnormality on chest film	13 (32 %)	10 (25 %)
Arterial acid-base status on admission		
pH	7.311 \pm 0.140	7.329 \pm 0.090
Base Excess mEq/l	-7.2 \pm 5.5	-7.2 \pm 4.0
Pco ₂ mmHg	44.2 \pm 17.9	37.7 \pm 14.2

^a According to the single birth intrauterine weight chart of Lubchenco et al (25)

^b Known in 32 infants

^c Known in 31 infants

(Pco₂) oxygen tension (Po₂) and pH were corrected for the temperature of the baby. Additional details on methods of sampling measurement and calculation have been described elsewhere (6, 37).

In some of the infants on the second day of life several blood components (serum Na, K, Ca, P and BUN) and the ECG were studied simultaneously (i.e. within a time lapse not longer than 2 hours). In order to avoid hemolysis blood samples were taken in a plastic syringe allowed to clot into a test tube under mineral oil and centrifuged at slow speed. Serum Na and K were measured by flame spectrophotometry, serum Ca by the method of Kramer & Tisdall (22) in the first part and by the method of McPherson (30) in the last part of the investigation (results by both methods were found to be reasonably reproducible), serum P by the method of Fiske & Subbarow (13), the blood urea nitrogen (BUN) by the method of Fawcett & Scott (12). The ECG was taken with a direct writing machine recording the usual leads (I, II, III, aVR, aVL, aVF, VR, V, V, V₆). Low voltage was frequently found and in such cases a standardization of 1 mV = 20 mm was used.

The total serum bilirubin was determined daily (and more frequently in the presence of rapidly increasing or dangerous bilirubin levels) starting from the time when a significant jaundice was clinically evident and until a definite decrease of serum bilirubin was observed. The method of Malloy & Evelyn (27) was used. Direct reacting bilirubin was determined at least once in subjects with total serum bilirubin higher than 17 mg/100 ml. The hematocrit was

determined on arterial or arterialized capillary blood after centrifugation in capillary tubes at 4000 rpm for 5 min.

Additional details on plans and methods of the investigation have been reported elsewhere (32).

RESULTS

Findings on admission and comparability of experimental groups

The trial was performed from December 1965 to July 1968. Some characteristics and some findings on admission in the treatment and in the control groups have been reported in Table 1. The difference between the experimental groups were small and statistically not significant. Additional information on clinical and laboratory findings on admission and on the amount of fluid actually administered has been given in another report (32).

Post-natal changes of acid-base parameters

In the first 10 days of life 257 pH, Base Excess (BE) and Pco₂ determinations were performed on arterial (97%) or arterialized capillary (3%) blood. In addition 20 BE determinations were performed on capillary or venous blood. On this data, the post-natal changes of acid-base parameters in treated and control infants were investigated. However in view of the heterogeneity of clinical and pathophysiological conditions encountered in the present series, it was considered appropriate to analyse the data separately in subjects with or without marked abnormality on chest film.

Results have been shown in Figs 1 to 3. On the average acidemia was present in the early hours of birth, more markedly in babies with severe X-ray abnormality. In the first 2 days of birth the acid-base parameters showed minor changes in the controls, whereas in treated infants the Base Deficit decreased and the pH increased. After the 3rd day of life the pH was on the average lower in treated infants because of markedly higher Pco₂ levels.

Serum electrolytes and ECG findings in the 2nd day of life

During the 2nd day of life blood chemistry studies (serum Na, K, Ca and P, BUN) were performed in 19 treated and 23 control in

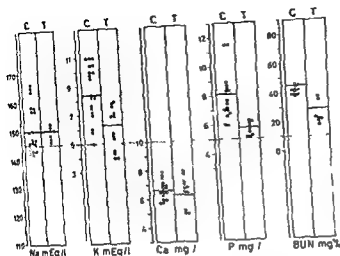


Fig 4 Blood chemistry findings in the 2nd day of life in treated (T) and control (C) infants considered in Table 2. Horizontal bars represent mean values and the dotted line the mean normal level.

infants who survived for at least 5 days (in no case direct bilirubin exceeded 10% of the total). Thus only the latter group of infants was considered.

Treated and control subjects were closely comparable with respect to mean birthweight, gestational age, incidence of RDS in the first two days of birth, and incidence of ABO incompatibility with the mother's blood. Treated infants received about twice as much fluid as the controls in the first 3 days of life and about 20% more in the 4th-5th day. The incidence of hyperbilirubinemia (both ≥ 17 mg/100 ml and ≥ 20 mg/100 ml) was remarkably but not significantly ($p=0.06$ and $p=0.10$ respectively) higher in the control group. There was a trend for hyperbilirubinemia to occur earlier in control than in treated infants; in fact, when considering control infants, hyperbilirubinemia was first found in 4 cases in the 4th day and in 3 cases in the 5th-6th day, whereas in treated infants it was first discovered in the 4th day in one and in the 6th day in 2 cases. In the treatment group, the highest total serum bilirubin observed was of 20.2 mg/100 ml, and the infant recovered spontaneously. Four controls had peak total serum bilirubin between 22 and 26 mg/100 ml and received one or two exchange transfusions. In 17 treated and 13 control infants, determinations of arterial hematocrit both in the 1st and in the 4th-5th

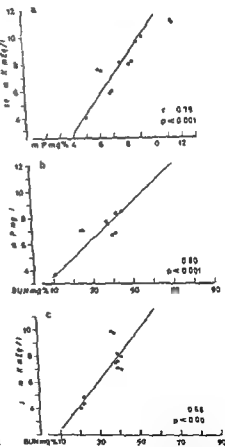


Fig 5 Correlation between serum K, serum P and BUN levels in the 2nd day of life in treated (●) and control (○) infants considered in Table 2. The correlation coefficient r , the regression line, and the statistical significance of correlation are also shown.

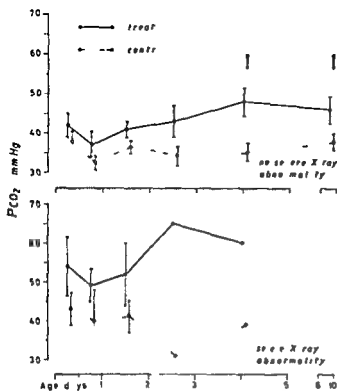


Fig 3 Mean \pm S.E. of arterial P_{CO_2} in treated and control infants. Other explanations as in Fig 1.

was calculated roughly the same proportion of values from the two groups was located above or below the line. No correlation whatsoever was observed between serum Na or serum Ca on the one hand and the remaining blood findings in the first or second day of life on the other.

In part of the infants with blood chemistry studies the ECG was simultaneously taken (Table 3) showing in about one third of the controls prolonged PR interval, QT interval and QRS complex. A QT interval longer than 0.40 sec was found in 7 controls and was associated in 6 instances with bradycardia due to 2:1 atrio-ventricular block (7). With the exception of one infant with a PR interval of 0.16 sec such findings were never observed in the 16 treated patients with ECG studies.

The ECG abnormalities were always associated with high serum K values, in particular all subjects with QT interval longer than 0.40 sec had serum K higher than 9.5 mEq/l. After pooling the results from treated and control patients no significant correlation was calculated

between the serum K level and the duration of the QT interval (Fig 7). On the contrary, no correlation was present between the serum Ca level and the duration of the ECG parameters considered.

The six controls with 2:1 atrio-ventricular block received variable amounts of 1% CaCl₂ intravenously, and all of them recovered.

Hyperbilirubinemia (Table 4)

Hyperbilirubinemia (i.e. peak total serum bilirubin ≥ 17 mg/100 ml) was never observed in the 31 infants (12 treated and 19 controls) who lived less than 72 hours, nor in the 12 infants (8 treated and 4 controls) who died in the 4th or 5th day of birth. On the other hand hyperbilirubinemia was found in 10 of the 37

Table 2 Data on admission and findings in the 2nd day of life (mean \pm S.D. or %) in part of treated and control infants in the trial. (Numbers in parentheses indicate the number of observations when different from the total number)

	Treated	Controls
Number of cases	19	23
Data on admission		
Weight kg	1.05	1.10
Gestational age weeks	28.4 (14)	27.8 (20)
No. () & marked X ray abnormality	1 (5)	4 (17)
arterial pH	7.31	7.35
Blood studies on 2nd day		
Serum Na mEq/l	149.7 \pm 12.4 (16)	150.2 \pm 8.8 (27)
Serum K ^a mEq/l	6.3 \pm 1.8 (18)	8.4 \pm 2.0 (27)
Serum Ca mg/100 ml	6.4 \pm 1.3 (16)	6.6 \pm 0.8 (71)
Serum P ^a mg/100 ml	5.8 \pm 1.2 (14)	8.1 \pm 2.0 (19)
BUN ^a mg/100 ml	29.2 \pm 15.2 (15)	44.6 \pm 14.9 (15)
Arterial Ht difference ^a (1st-2nd day)	5.8 \pm 8.3 (16)	0.4 \pm 5.3 (15)
Therapy		
No. () & servo contr. of body temp	8 (42)	8 (35)
Mean amount of fluid (ml) given before blood studies	56 ^b	13.5 ^c

^a Significance of difference between treated and controls $p < 0.01$.

^b Only 1 v. oral feeding never given.

^c Only oral 10% gluc. milk given to one baby only.

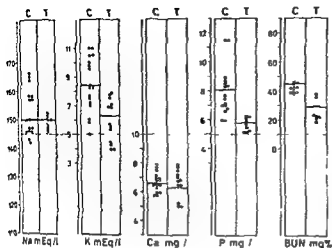


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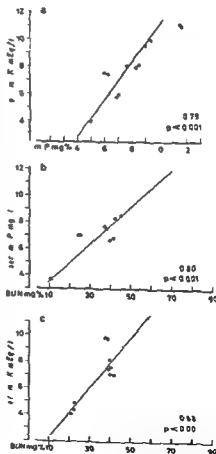


Fig 5 Correlation between serum K, serum P and BUN levels in the 2nd day of life in treated (●) and control (○) infants considered in Table 2. The correlation coefficient = the regression line and the statistical significance of correlation are also shown.

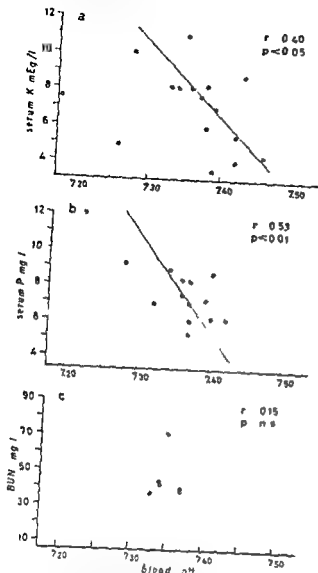


Fig 6 Correlation of arterial pH with serum K, serum P and BUN (all results in the 2nd day of life). Other explanations as in Fig 5.

Table 3 ECG findings in the 2nd day of life in part of babies considered in Table 2

	Treated	Controls
Number of cases	16	21
No () of PR int > 0.12 sec	1 (6)	6 (32)
No () of QRS int > 0.04 sec ^a	0 (0)	8 (39)
No () of QT int > 0.40 sec ^b	0 (0)	7 (33)
No () of 2:1 atrioventricular block ^b	0 (0)	6 (29)

^a ^b Significance of difference between treated and controls ^a $p < 0.01$ ^b $p < 0.02$

^c On 19 tracings data could not be calculated on 2 tracings with 2:1 atrioventricular block

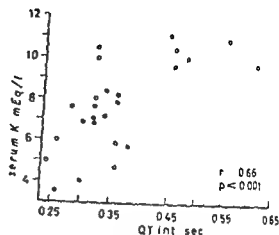


Fig 7 Correlation between serum K and QT int $r = 0.66$ $p < 0.001$ in the 2nd day of life. Other explanations as in Fig 5.

day of life were available. On the average, the Ht drop was only slightly greater in treated than in control infants.

DISCUSSION

Interpretation of results

In the present study the management of treated and control infants differed by three respects: oral feeding or i.v. infusion anti-

Table 4 Some findings (mean or %) relevant to the problem of hyperbilirubinemia in treated and control babies who lived for at least 120 hours

	Treated	Controls
No. of cases	20	17
Birthweight kg	1.10	1.11
Gestational age weeks	28.3 ^b	29.1 ^c
No () of RDS ^a	11 (55)	7 (41)
Fluid (i.v. and/or oral) given		
days 1-3	220	102
days 4-5	259	214
No () of ABO incompatibility	3 (15)	3 (18)
No () of peak total serum bilirubin		
> 17 mg/100 ml	3 (15)	7 (41)
> 20 mg/100 ml	1 (5)	4 (24)
No () of exchange transfusion	0 (0)	4 (24)
Arterial Ht difference (1st-4th/5th day)	8.1 ^d	4.8

^a RDS = Silverman's score ≥ 2 and/or resp rate > 60 /min for at least 48 hours after birth

^b On 17 infants

^c On 16 infants

^d 17 observations

^e 13 observations

microbial regimen administration of fresh human plasma. However there is no reason to suspect that the different antimicrobial regimens or the plasma administration could affect the biochemical or cardiac findings reported in the present paper. Therefore in the discussion different results in the experimental groups will be considered as related to oral feeding or to IV infusion with water, glucose and NaHCO_3 .

Metabolic effects of fasting and early iv infusion

In agreement with previous studies on infants with comparable weight, postnatal age and therapy (3, 35, 49) the present investigation showed conclusively that fasting newborns with very low weight often develop shortly after birth very high levels of serum K and P and of BUN which can be prevented at least in part by early infusion therapy. Hyperkalemia and different serum K levels between infants with early infusion or routine oral feeding were not observed in another trial in the 4th day of life (11). In order to explain such discrepancy the different ages of patients studied should be taken into consideration as well as the different risk of hypoxia and hypothermia prior to admission in extramurally or intramurally born infants.

Increased tissue catabolism and decreased renal function due in turn to prematurity, fasting, perinatal stress and asphyxia have been postulated to explain these findings and have been substantiated by balance studies in fasting and early fed newborns (7, 3, 29, 35, 53, 55). The present data do not bring direct evidence on the etiology of these abnormalities but emphasize the role of extreme prematurity and of starvation. In addition the close correlation between changes of serum K, serum P and BUN (Fig. 7) suggests that these abnormalities share to a great extent the same origin. The weak (but still significant) inverse correlation of blood pH with serum K and P (not with BUN) suggests that an additional mechanism related to acidosis plays a role in the origin of hyperkalemia and hyperphosphatemia

(but not of hyperazotemia). The regression lines calculated from the present data showed a decrease in serum K of 4.7 mEq/l and in serum P of 5.0 mg/100 ml , per unit pH increase. These values were somehow intermediate between the coefficients of 3 and of 6 found experimentally in uncompensated acidosis (respectively respiratory and non respiratory) in the dog (44). Among the mechanisms involved in these changes the release of intracellular K in exchange with H (10) and splitting of organic phosphate compounds due to a fall in intracellular pH (16) must be considered.

As to hyperbilirubinemia reports on the effect of early as compared to late fluid and calories administration on serum bilirubin levels have been contradictory and prevention of severe hyperbilirubinemia has been observed by some authors (19, 24, 45, 52, 56) not by others (4, 11, 17, 20, 28). The present results were in agreement with the former point of view but additional investigation possibly taking into account the many factors involved in the etiology of hyperbilirubinemia of prematurity (26, 36) is necessary. It may be questioned whether the decrease in the incidence of hyperbilirubinemia after iv infusion was due to a beneficial metabolic effect or to hemodilution. In the present series hemodilution as indicated by the drop of hematocrit after the day of birth in treated babies as compared with controls was on the average fairly marked in the second day of life (Table 2) but rather limited in the 4th-5th day (Table 4) when marked hyperbilirubinemia was first found to occur. For this reason and in consideration of the large extravascular pool of bilirubin in hyperbilirubinemic infants (51) it seems unlikely that hemodilution might have contributed significantly to the decrease in the incidence of hyperbilirubinemia after iv infusion.

Blood glucose determinations were not performed in the present study. As reported elsewhere (32) the incidence of clinical signs usually associated with hypoglycemia (such as apnoeic spells, increased muscular tonus, tremors

and cloni (38)), was not significantly different in the present series of babies with or without iv infusion, although cloni and increased tonus were moderately more frequent in fasting subjects

Early neonatal hypocalcemia

It has been previously shown that in newborns with low weight serum Ca levels decrease in the early days of birth, and that this decrease is greater the lower the weight (5, 14, 47). Therefore, the very low serum Ca values observed in the present series in the second day of life were not surprising. The pathophysiology of early neonatal hypocalcemia is largely unknown (34). In a recent investigation (47) early neonatal hypocalcemia was found to be associated with birth asphyxia, prematurity, respiratory distress, increased serum P and decreased blood pH and serum protein in the early hours of birth and with NaHCO_3 administration. However the cause-effect relationship of these findings with hypocalcemia was not clear. The present investigation, performed on subjects with a very narrow birthweight range, showed that serum Ca levels were not related to serum K and P, to BUN or to blood pH levels and were similar in infants with or without iv infusion. This suggests that early neonatal hypocalcemia, although related to prematurity and possibly also to perinatal distress, was due to mechanisms differing from those involved in the serum electrolyte changes previously discussed and also that it was not secondary to the other blood chemistry abnormalities considered in the previous paragraph. In addition it was shown that the administration of NaHCO_3 was not a cause of early hypocalcemia. The possible relationship of hypocalcemia with ECG abnormalities will be discussed below. Other adverse effects possibly related to hypocalcemia were not apparent.

Electrocardiographic abnormalities

ECG abnormalities similar to those encountered in the present study have been previously

reported in newborns with very low weight, and have been attributed to hypocalcemia, hyperkalemia, or a combination of the two (7, 9, 15, 31, 33, 46, 48). The present data, as well as more extensive investigations from this Centre (31) indicated that such ECG changes were certainly related to hyperkalemia. However their relationship to hypocalcemia was uncertain. In fact, on the one hand the ECG abnormalities were always associated with hypocalcemia and could be reversed by the iv administration of Ca, on the other hand no correlation was found between degree of ECG abnormality and serum Ca levels. Thus it was not clear whether the effect of Ca administration was due to the correction of hypocalcemia or if it was rather to be considered as a pharmacological effect related to Ca-K interaction in myocardial excitability and contractility (8, 18). Further studies including the determination of ionized Ca are needed to elucidate this problem.

Acid base status

In a previous trial on infants with HMD at birthweight of 1.25–2.5 kg (41) patients given iv infusion with glucose and NaHCO_3 showed when compared to patients with or without feeding higher Base Excess and blood pH values in the first two days of birth and similar blood pH but higher Pco_2 levels thereafter. In the present series of infants with low weight and with or without pulmonary disease a similar pattern was observed except that in the treated infants the late hypercarbia was more marked so that the mean blood pH was lower than in the controls. Although such late hyperventilation after glucose and NaHCO_3 administration was not associated with an increased incidence of apnoeic spells (32) this finding should be regarded with some concern and further trials contrasting relatively liberal or restricted policies of NaHCO_3 administration after the first 2 or 3 days of birth seem indicated.

Indications and policy of early iv infusion with glucose and NaHCO₃ in newborns with very low weight

In another report on the present series of newborns with very low weight (32) no difference in neonatal survival after oral feeding or iv infusion was observed. On the other hand the present report in keeping with studies discussed above has shown that early acidemia, hyperkalemia, hypozotemia, hyperbilirubinemia and ECG abnormalities were prevented or corrected at least in part, by the early iv infusion. Other investigations have shown that hypoglycemia frequently occurs in fasting newborns with low weight and can be prevented by early oral or iv feeding (4, 11, 17, 28, 38, 39, 43, 56). It seems reasonable to suspect that in newborns with optimal perinatal care (such as subjects immediately transferred from the delivery room to the Intensive Care Unit under well controlled conditions) the incidence of such abnormalities would be lower than in the present series of extramurally born patients. However, since many of these abnormalities are potentially dangerous to the central nervous system, it is suggested that while waiting for a final evaluation by long term follow up studies in survivors, the early iv infusion therapy should be given routinely to newborns with very low weight.

Nevertheless, unnecessary and potentially dangerous therapeutic procedures should be avoided. The present observations have shown that correction of acidemia was achieved by the iv infusion therapy in the first 2 days of birth, but that subsequently marked hypovenilation occurred. The beneficial effect on serum electrolytes and ECG parameters was already obtained after few hours of infusion. The decrease in the incidence of hyperbilirubinemia was already apparent in the 4th day of birth. It seems therefore that the full benefit of early iv infusion with glucose and NaHCO₃ was in most cases obtained within the first 2 or 3 days of therapy. On the other hand, central thrombosis and hepatic lesions after umbilical vein catheterization have been reported with in-

creasing frequency (40, 42, 54) and it has been shown that the incidence of such lesions increases markedly after 48 hours of catheterization (23). It is therefore suggested that unless uncommon problems are present, the iv infusion therapy should not be administered for more than 2 or 3 days, possibly associated with a fast oral feeding schedule which has been shown to be feasible in newborns with very low weight (11, 45).

SUMMARY AND CONCLUSIONS

A controlled therapeutic trial was performed in newborns with birthweight of 750-1250 g. 40 infants (the treatment group) received an iv infusion with glucose and NaHCO₃ from the 1st to the 4th-7th day of life and increasing amount of human milk from the 2nd day of life. They also received in the newborn period 3 antibiotics (penicillin, methicillin and colistin), gamma globulin and fresh human plasma. 40 infants (the control group) received only oral feeding with 10% glucose and human milk starting in the 2nd day of life and 1 m kanamycin.

As compared to controls, in treated infants acidemia decreased more rapidly in the first two days of birth, but hypercarbia developed after the 3rd day.

Blood electrolytes and ECG studies were performed in part of the infants in the 2nd day of life. Markedly increased serum K and P and BUN levels were observed in controls and significantly lower values in treated infants. Severe ECG abnormalities were associated with hyperkalemia in controls and were never observed in treated infants. Serum Ca levels were low and similar in the two groups. Hyperbilirubinemia occurred more frequently in controls and exchange transfusion was performed only in this group of infants.

Since many metabolic abnormalities commonly occurring in fasting newborns with very low weight are potentially dangerous to the central nervous system, the conclusive evaluation of the iv infusion with glucose and

and cloni (38)), was not significantly different in the present series of babies with or without iv infusion, although cloni and increased tonus were moderately more frequent in fasting subjects.

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NaHCO_3 must await long term follow up studies in survivors. While waiting for this evidence, it is suggested that this therapy should be given routinely to newborns with very low weight. The present observations also suggest that, if a reasonably fast oral feeding schedule is provided, in most circumstances the infusion therapy should not be continued after the 3rd day of birth.

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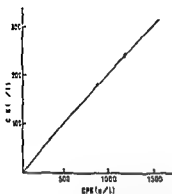


Fig 1 Comparison of two methods used for the determination of serum creatine phosphokinase. Various sera were simultaneously analysed for CPK activity as mentioned under Methods. The results obtained by the method of Okinaka et al (7) are plotted along the ordinate; the results obtained by the method of Rosalki (11) along the abscissa.

CoQ (hexahydrocoenzyme Q₁₀ 20 mg/ml or 100 mg/ml in corn oil) was supplied by one of us (K.F.). In the case of the twins the control patient received commercial corn oil.

CASE HISTORIES AND RESULTS

Case 1 and 2

K. H. and K. S. are identical male twins born May 27 1961. The family history was non-contributory. They could sit unsupported when 8 months old and walked without support at the age of 16 months. Walking difficulties soon became apparent. By admission to our hospital (March 15 1968) at the age of 7 years they walked with a pronounced waddling and supported both hands on the knees when getting up from the prone position. The muscles of the lower extremities and back were weak and the calves showed pseudohypertrophy. The diagnosis of muscular dystrophy was confirmed by typical histological features in muscle biopsy and by typical findings on electromyography. There were raised levels of several serum enzymes (GOT 63 and 99 U/l; GPT 60 and 10 U/l; LDH 840 and 1060 U/l; CPH 368 and 394 U/l for K. H. and K. S. respectively).

CoQ treatment was started in May 1968 and initially the subjects were kept on a daily dose of 50 mg. In September 1968 there was no change in the clinical picture therefore the daily dose was increased to 150 mg but without effect. Fig. 2 shows the results of a therapeutic study starting in March 1969 and lasting 4 months. Before the experiment K. S.

and K. H. were both on 150 CoQ daily (small dose). At this time both had markedly elevated activities of CPK. During the period of treatment K. S. received 1 g CoQ daily (large dose) while K. H. received corn oil only. There was a marked decrease of CPK in subject K. S. during the period of treatment. However there was an even more pronounced decrease in the control subject. Fig. 2 also shows the results of the aldolase determinations. At the beginning of the experimental period both patients had elevated activities of serum aldolase with the higher activity in the control subject. At the end of the experiment the control subject K. H. showed an unchanged aldolase activity whereas there was a statistically insignificant increase in the patient receiving CoQ. In July 1969 there was a definite decline in their motor power and they were no longer able to walk even with support. CoQ treatment was discontinued in August 1969.

Case 3

This boy (G. S.) was born March 11 1961 and was first admitted to our hospital on April 18 1967. The family history was non-contributory. He could sit without support when 7 months old and walked unsupported when he was 16 months but showed early difficulties in running and stairclimbing. On admis-

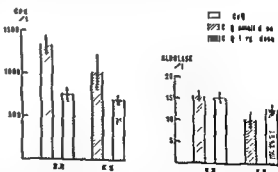


Fig 2 Creatine phosphokinase and aldolase activities in serum in a pair of identical twins. Before the experiment both twins were given 150 mg CoQ daily (small dose). During an experimental period of 4 months subject K. S. received 1 g CoQ daily (large dose) while subject K. H. received corn oil only. The enzyme activities were determined as described under Methods, the method of Rosalki (11) being used for CPK-determination. Each bar represents the mean of 4-6 determinations and the standard error of the mean is indicated on the top of each bar.

COENZYME Q IN DUCHENNE MUSCULAR DYSTROPHY

A Preliminary Therapeutic Trial

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The muscular dystrophies which give rise to progressive weakness and wasting of skeletal muscles, represent a great challenge in current medical research. Since no drug treatment is at present known which can change the course of the disease in any form of muscular dystrophy (18) it became of interest to study the effect of Coenzyme Q (CoQ). CoQ which is part of the electron transport system in mitochondria (9) exerts vitamin activity in the rabbit with nutritional muscular dystrophy (17, 14). Intravenous administration of CoQ to these animals had a favourable therapeutic effect and was even lifesaving. A less dramatic effect was observed in mice with a genetic type of dystrophy. Thus Farley et al (2) observed physical improvement in genetically dystrophic mice after administration of CoQ. Later Scholler et al (12) were able to demonstrate physical improvement as well as extension of life in these animals when given CoQ orally.

In the present work we report a study on 4 boys with a Duchenne type of muscular dystrophy. Two of these boys were identical twins and they offered an unusual opportunity for a controlled clinical experiment. The patients were all followed during a period of more than 1 year. Apart from a clinical evaluation we were in particular interested in the serum levels

of creatine phosphokinase (CPK) and aldolase. The serum levels of these as well as of several other enzymes, are markedly elevated in muscular dystrophy of the Duchenne type (16).

METHODS

For the enzyme assays heparin blood was collected in the morning with the patient fasting and at rest. The plasma was kept on ice until analysed. CPK was initially determined as described by Okinaka et al (7) the phosphate produced being determined by the method of Fiske & Subbarow (3). Reduced glutathione was present in the incubation mixture. Reagents produced by Sigma Chemical Company St Louis USA were used (Sigma Technical Bulletin No 661). The UV method of Tanzer & Gilvarg (15) at 25 served as reference method and normal serum values for male adults are below 12 U/l and for female adults and children below 8 U/l. Later CPK was determined at 25 by the method of Rosalki (11) using reagents produced by Calbiochem, Luzern, Switzerland. Normal serum values are below 35 U/l and 20 U/l for male and female adults respectively with the mean value for children of 2 to 10 years of age slightly below that for female adults (19). There appears to be a linear relationship between the two methods (Fig 1) the CPK activities obtained by the method of Rosalki (11) being higher with a factor of 5 as compared with the method of Okinaka et al (7). Aldolase was determined by the UV method of Racker (8) at 25 using reagents produced by Boehringer Mannheim, Germany. Normal serum values for adults are below 6 U/l and for children below 3 U/l (4). The transaminases were assayed by the method of Reitman & Frankel (10) and lactic dehydrogenase (LDH) by the UV method of Wroblewski & LaDuc (20) at 25.

ing itself in improved muscular strength (b) Reduced activities of CPK and aldolase which are serum enzymes with markedly increased activity in Duchenne muscular dystrophy

Concerning a clinical effect the therapeutic trial with the identical twins seems conclusive. Thus both twins became clinically worse and stopped walking at the same time, in spite of the fact that one received 1 g CoQ daily whereas the other received corn oil only. Of the 2 other patients studied one stopped walking while being treated like the twins where the other did not present any definite clinical change. It appears therefore that oral administration of this particular form of CoQ does not affect the fundamental disturbance in Duchenne muscular dystrophy.

Before concluding that there is no deficiency of CoQ in Duchenne dystrophy two important questions should be answered: 1. Does hexahydrocoenzyme Q₁ substitute for coenzyme Q₉ and Q₁₀ which are the main forms of the vitamin biosynthesized in mammalian tissues? 2. Does orally administered CoQ reach its place in the mitochondrial membranes of skeletal muscles?

It is of basic significance that hexahydrocoenzyme Q₁ has *in vitro* activity in the succinoxidase and the NADH oxidase systems. Lenaz et al. (6) found the activity of hexahydrocoenzyme Q₁ to be comparable to that of coenzyme Q₁₀ in the succinoxidase system and slightly less than that of coenzyme Q₁₀ in the NADH oxidase system.

The vitamin activity of hexahydrocoenzyme Q₁ was first demonstrated in the nutritionally dystrophic rabbit where oral administration was lifesaving (14). By contrast the rabbit failed to recover after oral administration of coenzyme Q₁₀, which is the homologue normally present in rabbit tissue (1). These observations clearly demonstrated that to obtain a therapeutic response *in vivo* transport requirements as well as structural requirements have to be met. Although hexahydrocoenzyme Q₁ appears to reach its enzyme site after oral administration in the rabbit, this might not be

the case in humans. And even if hexahydrocoenzyme Q₁ reached the skeletal muscle mitochondria it might not have the structural specificity necessary to restore a possible inadequate electron transport in Duchenne dystrophy.

It is generally agreed that the activity of CPK in serum is elevated in most cases of Duchenne dystrophy and that this enzyme is a sensitive parameter for the activity of the disease. Thus Goto et al. (5) found the serum levels of CPK to be related not only to the age of the patient and the duration of the disease but also to the progression and the severity of the dystrophy. It appears therefore that an improvement as well as a deterioration of Duchenne dystrophy might lead to only moderately elevated activities of CPK in serum.

As shown in Table 1 a rather marked decrease of CPK was observed in the subjects O S and J H J during treatment with CoQ. There was no corresponding clinical improvement. On the contrary the clinical condition of O S deteriorated during this period whereas the disease was rather stationary in the case of J H J. The plasma CPK of O S seemed to vary independently of the dose of CoQ (Fig. 3) showing a net decrease during the period of treatment. Also in the twins a decrease in CPK activity was observed. This decrease which was associated with reduced physical ability was of a similar order of magnitude in the treated patient and the control. It is therefore most likely that the decrease in plasma enzyme activities observed are coincidental and not caused by the CoQ treatment. A possible exception is subject J H J whose clinical condition was rather stable during the test period and where the decrease in CPK and aldolase activities cannot be attributed to a change in the activity of the disease.

Further clinical studies should be performed when other forms of the coenzyme are available. The particular derivative of CoQ used in the present study was well tolerated and gave no side effects even at high doses.

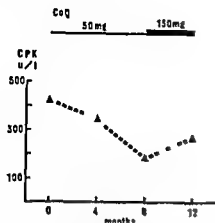


Fig. 3 Creatine phosphokinase during 1 year with CoQ treatment. Subject Ø S was given 50 mg CoQ daily for 8 months and then 150 mg daily for 4 months. He was admitted to the hospital at 4 monthly intervals. The points represent the mean of 5–12 determinations on separate days. CPK was determined by the method of Okinaka et al. (7).

sion he walked with a pronounced waddle and supported both hands on the knees when getting up from the prone position. The muscles of his hips and shoulders were weak and there was pseudohypertrophy of his legs. The diagnosis of muscular dystrophy was confirmed by electromyography and by examination of serum enzymes. GPT showed an activity of 128 U/l, GOT 91 U/l and LDH 740 U/l. CPK was not examined at the first admission.

CoQ treatment was started February 1968. Fig. 3 shows the CPK levels during treatment with 50 mg and later 150 mg per day. During the first 8 months his CPK dropped substantially from more than 400 U/l to about 200 U/l. His clinical condition however remained unchanged. After 8 months the daily dose was increased to 150 mg. During another 4 months there was no improvement in the clinical condition and CPK increased to slightly less than 300 U/l. When seen as an

outpatient in July 1969 there was a marked decline in the motor power. Thus from May 1969 he had not been able to walk. CoQ was discontinued in August 1969.

Case 4

This boy (J H J) was first seen June 1968 aged 11 years and 2 months. The family history was non-contributory. He could sit without support when he was 7 months, and walked at 12 months of age but fell quite easily and had difficulties in getting up from the prone position. He was able to climb stairs, dress himself, swim and ride a bicycle but continued to present muscular weakness. At examination he walked with a waddle and with a slight drop-foot on both sides. His skeletal muscles were generally weak with rather marked pareses of the hip muscles. The electromyography pattern was in agreement with a myogenic affection. Muscle biopsy showed atrophic muscle fibres. GOT, GPT and LDH were within normal limits whereas the CPK activity of serum was elevated (197 U/l).

Treatment with CoQ was started shortly after the first admission, and he was treated with increasing doses. After an initial improvement in his muscular strength his condition remained rather stable and in October 1969 he could still climb the stairs.

The activities of CPK and aldolase in serum of the patients Ø S and J H J kept on CoQ 1 g daily, are shown in Table 1. There was a marked decrease in the activities of both enzymes during a 4 month period. In the case of Ø S the decrease is statistically significant for both enzymes.

DISCUSSION

In the present work we were looking in particular for (a) A clinical effect of CoQ manifest

Table 1 Creatine phosphokinase and aldolase activities in plasma during treatment with CoQ

At the beginning of the test the CoQ dose was increased from 150 mg to 1 g per day. After 4 months on this dose the subjects were readmitted for clinical and laboratory evaluation. The enzyme activities were determined before and after the 4 month period.

Subject	Creatine phosphokinase u/l plasma ^a		Aldolase u/l plasma ^a	
	Before	After	Before	After
Ø S	1298 ± 45 (6) ^b	829 ± 87 (5) ^b	240 ± 40 (6)	114 ± 12 (4) ^c
J H J	872 ± 100 (5)	285	181 ± 49 (5)	89

^a ± SEM ^b $p < 0.001$ ^c $p < 0.02$

PERINATAL ASPHYXIA AND RESIDUAL PLACENTAL BLOOD VOLUME

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The present study was undertaken to determine the effect of perinatal asphyxia on the distribution of blood volume between the fetus and placenta. At present, it is unknown whether asphyxia results in pooling of blood in the placenta or transfer of placental blood to the fetus.

MATERIAL AND METHODS

Thirty-two vaginally delivered term infants were evaluated clinically by means of one minute Apgar score. The infants had their umbilical cords clamped as quickly as possible at birth, and in all instances prior to the first breath. A segment of cord was removed between two clamps for umbilical arterial blood sampling. The arterial blood was drawn into a heparinized syringe which was then immediately placed in ice. The blood was analyzed for pH using a Radiometer micro blood gas apparatus.

An additional clamp was left on the umbilical cord 4 d, after delivery of the placenta, it was dried quickly to remove maternal blood from its external surface. The placenta was then suspended in a funnel and the blood drained into a graduated cylinder as described by Redmond et al (6). The placenta was left in the funnel until spontaneous drainage ceased and, before removal, gentle pressure was applied over the maternal surface to express the last small quantity of placental blood. The volume of blood which drained into the cylinder was expressed in ml per kg body weight of the infant. Hematocrit was measured in cord venous blood and in capillary blood obtained from the baby's heel at 4 hours of age. The heel was warmed for one minute prior to sampling.

This work was supported by PHS Grant RRO 5351-08 National Institutes of Health Bethesda Maryland, U.S.A.

RESULTS

Apgar scores for the 32 infants ranged from 2 to 9 with a median of 7. The mean cord arterial blood pH was 7.25 with a range of 6.96 to 7.38. Seven infants had Apgar scores of 2, 3 or 4. These 7 infants had a mean arterial pH 7.12 with a range of 6.96 to 7.27. Of these 7, 3 severely asphyxiated infants (Apgar score 2-3) had pH values of 6.96-7.04 which were markedly lower than any other values in the group. These data are illustrated in Fig 1 and agree with other studies of cord blood acid base balance (2). The mean residual placental blood volume (RPBV) was 32 ± 13 ml/kg body weight. The 3 infants with the lowest cord arterial pH values had RPBVs which averaged 20 ml/kg body weight, which is approximately 1 SD below the group mean. The relationship between cord arterial pH and RPBV is illustrated in Fig 2.

The mean change in hematocrit from birth to 4 hours was $+14\%$ with a range of -4% to $+30\%$. Two of the 3 most severely asphyxiated infants (Apgar 2-3 and pH 6.96-7.04) had hematocrit changes of $+30\%$ and $+23\%$ which were markedly greater than the mean. Only one other infant had an increase in hematocrit as large as that shown by these 2 infants. The relationship between cord arterial pH and change in hematocrit over the first 4 hours is shown in Fig 3.

SUMMARY

The effect of hexahydrocoenzyme Q₁ (CoQ) has been studied in 4 cases of Duchenne muscular dystrophy, including a pair of identical twins.

CoQ was given by the oral route in doses increasing from 50 mg to 1 g daily. In the case of the twins one received CoQ, whereas the other was given corn oil as a control.

During a period of treatment of more than one year no improvement in muscular strength was observed. On the contrary, there was a steady progression of the disease in 3 of the 4 cases with an identical clinical course in the twins.

No overall consistent decrease in CPK or aldolase activities was observed during the period of treatment, but there was a statistically significant decrease of CPK and aldolase for one boy.

The absence of overall beneficial changes during this particular trial might have been because (a) hexahydrocoenzyme Q₁ is intrinsically inactive in Duchenne dystrophy, (b) the dose level was too low, (c) the disease in these particular boys was too advanced.

Besides these limitations this trial also does not exclude the possibility that other homologs of the coenzyme Q group may have therapeutic benefit in human muscular dystrophy.

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Key words: Coenzyme Q, muscular dystrophy

PERINATAL ASPHYXIA AND RESIDUAL PLACENTAL BLOOD VOLUME

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The present study was undertaken to determine the effect of perinatal asphyxia on the distribution of blood volume between the fetus and placenta. At present it is unknown whether asphyxia results in pooling of blood in the placenta or transfer of placental blood to the fetus.

MATERIAL AND METHODS

Thirty-two vaginally delivered term infants were evaluated clinically by means of one minute Apgar score. The infants had their umbilical cords clamped as quickly as possible at birth, and in all instances prior to the first breath. A segment of cord was removed between two clamps for umbilical arterial blood sampling. The arterial blood was drawn into a heparinized syringe which was then immediately placed in ice. The blood was analyzed for pH using a Radiometer micro blood gas apparatus.

An additional clamp was left on the umbilical cord and, after delivery of the placenta, it was dried quickly to remove maternal blood from its external surfaces. The placenta was then suspended in a funnel and the blood drained into a graduated cylinder as described by Redmond et al (6). The placenta was left in the funnel until spontaneous drainage ceased and, before removal, gentle pressure was applied over the maternal surface to express the last small quantity of placental blood. The volume of blood which drained into the cylinder was expressed in ml per kg body weight of the infant. Hematocrit was measured in cord venous blood, and in capillary blood obtained from the baby's heel at 4 hours of age. The heel was warmed for one minute prior to sampling.

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RESULTS

Apgar scores for the 32 infants ranged from 2 to 9 with a median of 7. The mean cord arterial blood pH was 7.25 with a range of 6.96 to 7.38. Seven infants had Apgar scores of 2, 3, or 4. These 7 infants had a mean arterial pH 7.12 with a range of 6.96 to 7.27. Of these 7, 3 severely asphyxiated infants (Apgar score 2-3) had pH values of 6.96-7.04 which were markedly lower than any other values in the group. These data are illustrated in Fig 1 and agree with other studies of cord blood acid base balance (2). The mean residual placental blood volume (RPBV) was 32 ± 13 ml/kg body weight. The 3 infants with the lowest cord arterial pH values had RPBVs which averaged 20 ml/kg body weight, which is approximately 1 SD below the group mean. The relationship between cord arterial pH and RPBV is illustrated in Fig 2.

The mean change in hematocrit from birth to 4 hours was +14% with a range of -4% to +30%. Two of the 3 most severely asphyxiated infants (Apgar 2-3 and pH 6.96-7.04) had hematocrit changes of +30% and +23% which were markedly greater than the mean. Only one other infant had an increase in hematocrit as large as that shown by these 2 infants. The relationship between cord arterial pH and change in hematocrit over the first 4 hours is shown in Fig 3.

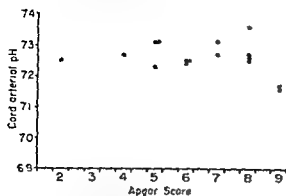


Fig 1 Apgar score at one minute of age and cord arterial blood pH

DISCUSSION

It has been reported that perinatal asphyxia results in arterial hypotension (4). This hypotension could, theoretically, be due to reduced blood volume. Redmond et al (6) presented data indicating that placental transfusion does not occur if the cord is clamped prior to the first breath. Thus hypotension might be expected in asphyxiated infants since early clamping is often carried out prior to the onset of respiration in order to facilitate resuscitation.

There are no direct data concerning the effect of fetal asphyxia on fetal placental blood volume distribution, although Dawes (1) showed that injection of epinephrine or nor-epinephrine in fetal lambs caused a decrease in fetal volume apparently due to a transfer of blood to the placenta.

On the other hand, Yao et al (9) studied blood volume in the first hour of life in normal and asphyxiated infants delivered by Caesarean section. The umbilical cords were clamped prior to extraction of the infant from the uterus. They found a mean circulating blood volume of 66 ml/kg in 13 normal infants and 90 ml/kg in 5 asphyxiated infants. One possible interpretation of these data was that asphyxia had caused a transfer of blood from placenta to fetus prior to delivery. A possible explanation for such a redistribution would be the greater tendency for the umbilical artery, by comparison with the umbilical vein to constrict in response to asphyxial stimuli (3).

Other explanations are possible for the blood volume data reported by Yao et al, as the authors themselves noted (9). It is possible that asphyxia resulted in an expansion of total circulating volume, rather than transfer from the placenta. Alternatively, asphyxia might have damaged capillary membranes and allowed leakage of the labelled albumin into the extra vascular space resulting in false measurement.

The present study is complimentary to the study of Yao et al (9), since we measured RPBV while they measured the infant's circulating volume. Our results suggest that asphyxia does result in transfer of blood from placenta to fetus or, in other words that the larger circulating volumes observed by Yao et

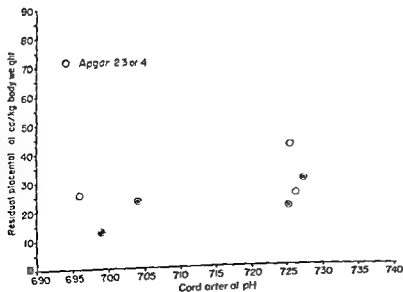


Fig 2 Cord arterial blood pH and RPBV. Infants with low Apgar score and low pH tend to have small RPBVs

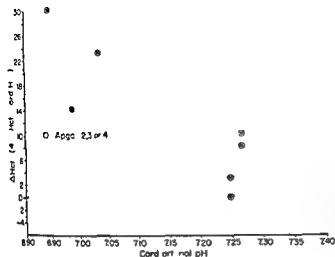


Fig 3 Cord arterial blood pH and increase in hematocrit from birth to 4 hours. Infants with low Apgar score and low pH tend to have large increases in hematocrit

al in asphyxiated infants were in fact due to transfer from placenta to fetus. We used cord arterial blood pH as a measure of perinatal asphyxia and related this observation to RPBV. Yao et al (8) recently observed that total fetal placental circulating blood volume at birth has a mean value of 105 ml/kg body weight with a very small range of distribution. Furthermore they demonstrated by clamping the cord at various intervals after birth, that as RPBV declines the infant's circulating volume increases by a like amount; the sum of the 2 fractions remaining constant whether the cord is clamped immediately at birth or 3 min later. Thus it is possible to measure RPBV and draw inferences from this measurement about the circulating blood volume of the infant.

Our mean value for RPBV was 32 ml/kg body wt, which is nearly identical with that reported by Yao et al (8) for infants with immediate clamping of the cord. In our 3 most severely asphyxiated infants the RPBV values of 12 to 25 ml/kg suggest by comparison with the data of Yao et al (8) that as much blood had already been transferred *in utero* as would ordinarily be transferred in the first 45 to 180 sec after delivery.

Two of the 3 infants with severe asphyxia and low RPBV's also had large increases in hematocrit over the first 4 hours of life. It

has been observed that a large placental transfusion results in a large increase in hematocrit over the first few hours of life due to a rapid decline in plasma volume (7). Therefore our data on hematocrit at birth and at 4 hours tend to support the concept that RPBV's were in fact low because placental transfusion had already occurred *in utero*. It is also possible however that impaired peripheral circulation was the cause for the higher capillary hematocrits in the more severely asphyxiated infants.

It would appear from our data that hypotension following perinatal asphyxia probably results from factors other than hypovolemia.

SUMMARY

The present data suggest that perinatal asphyxia may result in a transfer of blood *in utero* from placenta to fetus. The data contain no suggestion that asphyxia causes pooling of fetal blood in the placenta.

ADDENDUM

Philip et al (5) recently reported a study of RPBV in infants with early clamping of the umbilical cord. Unexpectedly low RPBV's were observed in infants with clinical signs of fetal distress in labor or low Apgar score one minute after delivery. Our data are in agree-

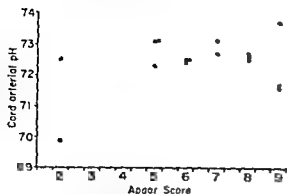


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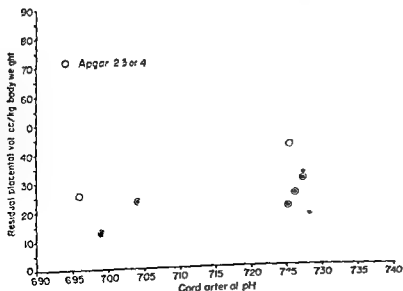


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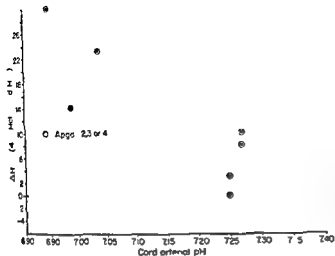


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Key words Perinatal asphyxia residual placental blood volume placental transfusion newborn

URINARY EXCRETION OF FREE AND CONJUGATED GLUCURONIC ACID IN JAUNDICED NEWBORN

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There is considerable variation in the intensity and duration of what has been called physiological jaundice in the newborn infant (5 10 13). It has been suggested by Matsuda & Shirahata (9) that lowered hepatic glucuronization as an expression of depressed enzymatic activity of the glucuronyl transferase system may play a role in many such examples of transient hyperbilirubinemia.

We set out to explore this possible correlation (without having recourse to biopsy study) between varying degrees of neonatal jaundice and the level of impairment or immaturity of glucuronyl transferase function. A significant parameter of the latter appeared to be expressed in the ratio between the urinary excretion of free and conjugated glucuronic acid

had begun to decline by the fourth day (4). In all 62 infants were examined 37 showing jaundice and 25 being non jaundiced.

In addition comparisons were made with the urinary excretion of free and conjugated glucuronic acid in the morning specimens of 13 premature infants and 15 healthy adult males. None of the subjects under study received any drugs.

Determination of glucuronic acid

The free and conjugated components of the total urinary glucuronic acids were determined by a modified version of the procedure described by Nir et al (12). Aliquots of urine (3-5 ml) were passed through a 9×100 mm Amberlite resin column. The resin CG4B 200 mesh was saturated and regenerated three times until finally prepared in its formate form. The columns containing the urine were washed with 100 ml distilled water which was discarded and then eluted with 100 ml aqueous 2 M NaCl.

The total glucuronic acid in the eluate was measured with naphthoresorcinol before and after treatment with sodium borohydride (3). In this way the free and conjugated glucuronic acid contents of the urine were determined. For each determination 5 ml of the eluate were treated with 1 ml of 0.4 M sodium borohydride and from this a 2 ml aliquot was used for the naphthoresorcinol reaction. The naphthoresorcinol reagent was prepared according to Nir (11).

MATERIALS AND METHODS

Normal samples of urine were collected from newborn male infants for the first 4 days of life and then preserved at 5°C. The urine was collected by attaching a small plastic bag to the penis and leaving it in situ for an hour or more until it contained sufficient urine for analysis. All urine samples were analyzed for free and conjugated glucuronic acid.

Serum bilirubin levels were simultaneously determined by the method of Malloy & Evelyn (8) on each of these 4 days and the infants accordingly classified into two groups "jaundiced" in whom the serum bilirubin level showed a continuing rise on the fourth day of life and "non jaundiced" in whom it

Supported by grant from the Ministry of Health Jerusalem

RESULTS

A significant difference ($p < 0.001$) in the ratio of free to conjugated glucuronic acid was found between the two groups. In the infants with increasing jaundice the free acid constituted about 55% of the total urinary glucuronic acid compared to 42% in the non jaundiced group (Table 1).

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though also decreased in newborn without manifest jaundice this output and its ratio with free glucuronic acid approaches the values of normal adults. It would appear that the physiological mechanism of conjugation is most depressed in premature and in physiologically jaundiced infants.

The low rate of excretion of urinary glucuronides could be due to the still incompletely developed glucuronyl transferase located mainly in liver microsomes and found to be chiefly responsible for glucuronide synthesis (7-14). Such reduced function of the newborn liver would be reflected in both low glucuronization capacity and a higher serum bilirubin level. A low urinary content of conjugated glucuronic acid is suggested as an indicator of this inadequacy of liver function in respect of the enzymatic activity of glucuronyl transferase.

Our findings would appear to be in accord with those in animal experiments demonstrating that liver glucuronyl transferase is relatively inactive at the time of birth (1-2, 7).

A reduced capacity to conjugate exogenous substances such as aminophenols and others has been defined in the newborn infant (15-16). The glucuronizing metabolism embracing these chemicals may not exploit the same pathways as that of endogenous substrates. It has been reported for instance that glucuronization of chloramphenicol is not impaired in premature infants with higher than normal bilirubin levels (6). Our studies indicate that in newborn infants especially premature and jaundiced a low glucuronization capacity is a characteristic reflection of their physiological immaturity of liver function.

Although a low urinary content of conjugated glucuronic acid fairly consistently co-existed with a high plasma bilirubin level jaundice was not always accompanied by decreased glucuronide excretion perhaps because the hyperbilirubinemia in some cases may predominantly derive from hemolytic element (4).

A high percentage of free urinary glu-

curonic acid would itself indicate depressed or pathological liver function at least in respect of its glucuronization capacity. The excretion rate of both free and conjugated urinary glucuronic acid should provide diagnostic information to help distinguish the underlying type of jaundice. Jaundice with a high free and low conjugated urinary glucuronic acid would indicate impaired liver function in this context. Indeed several preliminary tests in obstructive and hepatocellular jaundice have shown a low rate of conjugated urinary glucuronic acid to occur only in the latter type an aspect requiring confirmation on a larger scale.

SUMMARY

Free and conjugated components of the total urinary glucuronic acid were analyzed daily during the first 4 days of life in 62 newborn male infants. The newborn were divided into two groups, jaundiced 37 infants in whom the serum bilirubin level on the fourth day of life was either the same or higher than the level on the third day and "non jaundiced" 25 infants in whom the serum bilirubin level showed a fall on the fourth day after an initial rise during the first 3 days. The percentage of free glucuronic acid excreted was found significantly higher in the jaundiced infants.

Premature infants studied for comparison demonstrated an even higher percentage of free glucuronic acid excreted in the urine than the jaundiced infants. On the other hand in healthy adult males the percentage of free glucuronic acid excreted was found to be the lowest.

No significant difference was found in the total glucuronic acid concentrations of the jaundiced and non jaundiced infants.

It could be concluded therefore that when a high percentage of free glucuronic acid is excreted in the urine this probably indicates impaired conjugating capacity of the liver.

Table 1 The percentage of free glucuronic acid (free:total ratio=F/T) in the urine of various groups

Group	F/T \pm S.D. of the mean ()	Number in group
Premature (birth weight less than 2 kg)	66.6 \pm 9.8 \downarrow	13
Jaundiced newborn	55.1 \pm 6.4 \downarrow	37
Non jaundiced newborn	41.9 \pm 7.6 \downarrow	25
Adults	36.2 \pm 3.6	15

$\downarrow - p < 0.001$ $\downarrow - p < 0.01$

In the 13 premature infants run parallel for comparison 66% of the total urinary glucuronic acid proved to be free whilst only 36% did so in the 15 healthy adult males (Table 1).

The correlation between the percentage of free urinary glucuronic acid (F/T) and the serum bilirubin in the jaundiced and non jaundiced infants during their first 4 days of life is shown in Table 2.

In order to analyze the relationship of the ratio of free to conjugated urinary glucuronic acid between the jaundiced and non jaundiced infants the number of infants with F/T more and less than 50% was calculated in each respective group (Table 3).

It can be seen that on the first day of life there is already a difference between the F/T ratios of the jaundiced and non jaundiced in

Table 3 The percentage of free (F/T) urinary glucuronic acid of jaundiced (J) and non jaundiced (N J) newborn during the first four days of life

F/T	Day of life							
	1*		2**		3**		4**	
	J	N J	J	N J	J	N J	J	N J
>50	20	7	25	4	30	6	37	2
<50	12	16	11	20	6	17	4	10
Total no newborn	32	23	36	24	36	18	36	71

* $p < 0.05$ ** $p < 0.001$

infants the disparity becoming highly significant ($p < 0.001$) between second and fourth day of age, climaxing on the fourth day (Table 3).

The specific gravity of the urine samples was determined and no difference was found between the jaundiced and non jaundiced infants (1013-1016).

DISCUSSION

It is believed that the above results indicate that the measure of free and conjugated hexuronic acids of which glucuronic acid is the chief component, excreted in the urine is a relatively simple index of the efficiency of hepatic glucuronide synthesis in the newborn.

The output of conjugated glucuronic acid was found to be lowest in a group of premature infants yet almost as low in most newborn infants with physiologic jaundice. Al-

Table 2 Mean urinary glucuronic acid values (F/T) and serum bilirubin levels (S B) in jaundiced and non jaundiced newborn infants during the first four days of life

Day of life	Non jaundiced (25 infants) Glucuronic acid					Jaundiced (37 infants) Glucuronic acid				
	Free (mg/100 ml)	Conju- gated (mg/100 ml)	Total (mg/100 ml)	F/T ()	S B (mg/100 ml)	Free (mg/100 ml)	Conju- gated (mg/100 ml)	Total (mg/100 ml)	F/T ()	S B (mg/100 ml)
1st	10.6	12.9	23.5	45.1	1.22	9.2	9.1	18.3	50.3	1.78
2nd	10.7	15.9	26.6	40.2	2.76	11.5	10.0	21.5	53.5	4.23
3rd	8.0	10.1	18.1	44.2	3.76	10.8	8.6	19.4	55.7	6.60
4th	5.5	7.8	13.3	41.4	2.97	9.0	6.2	15.2	59.2	8.25

CHRONIC HYPOMAGNESEMIA WITH MAGNESIUM DEPENDENT HYPOCALCEMIA

1 A New Syndrome with Intestinal Magnesium Malabsorption

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A new form of tetany and convulsions with chronic hypomagnesemia and secondary hypocalcemia has been discovered in recent years (17 28 29 35 36 42)

The present study concerns another patient with this disease who was followed for 3 years¹

The clinical course will be briefly described and the results of many metabolic investigations will be presented and discussed. The results of these investigations were compared with those obtained in control children.

To evaluate better the influence of Mg deficiency on Mg metabolism we also studied a control boy fed with a low Mg and normal-Ca diet.

In a second paper the relationship between Mg and Ca/P metabolism will be taken into consideration.

CASE REPORT

A 7 months old Italian boy was admitted to the hospital with convulsions and typical signs of tetany. Irritability and psychomotor retardation were observed from the age of 4 months. Vitamin D and calcium therapy was previously prescribed at home without any effect.

This investigation was supported by grant 4580 from Swiss National Foundation for Scientific Research (fond National Suive de la Recherche Scientifique).

The case was presented for the first time at the Annual Meeting of the European Society for Paediatric Research (Vienna, August 1968).

Plasma calcium phosphorus and alkaline phosphatase were respectively 7.5 mg/100 ml 8 mg/100 ml and 48.8 i.u. (normal values 17 to 100 i.u.)

Tetanic EMG hypocalcemic ECG tracings and convulsive potentials on EEG were demonstrated. No X-ray findings of rickets were observed. Tibia and femur metaphysis appeared a little thickened.

The course of the disease is summarized in Fig. 1. No therapeutic effect was obtained with barbiturate intravenous Ca gluconate and oral CaCl₂ administration (total dose of Ca⁺⁺ 5.8 g during 8 days). At this time plasma magnesium was measured and was found to be low (0.8 mg/100 ml; normal values between 1.9 and 2.5 mg/100 ml). A diagnosis of hypoparathyroidism with possible Mg-deficiency (13) was made but prolonged therapy with i.v. Ca gluconate (total dose of Ca⁺⁺ 15.4 g) i.m. injection of Lilly[®] parathyroid extract (total dose 4130 i.u.) 10% MgSO₄ solution (total dose of Mg 2 g) and vitamin D (total dose of 7.5 mg) was only partially successful. On this therapy plasma calcium remained at subnormal levels (on only one occasion it rose to 9 mg/100 ml); plasma phosphorus remained normal but on two occasions it rose to 9 and 7.7 mg/100 ml; plasma magnesium never reached normal levels. Convulsions became less frequent but both tetanic manifestations and irritability remained unchanged.

Only a high oral intake of Mg glycerophosphate (3-7 g/day) completely normalized the clinical picture. ECG EMG EEG plasma calcium and phosphorus. Plasma magnesium rose (once up to 1.9 mg/100 ml) but practically never attained complete normal levels. Each time Mg supplementation was discontinued irritability tetany (once convulsions) EEG ECG EMG alterations and later hypocalcemia and hyperphosphoremia reappeared (Fig. 1). IQ at the age of 32 months was 68.

Diarrhoea frequently occurred both on normal diet and on Mg supplementation.

The patient's sister and parents have a normal plasma magnesium level.

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CHRONIC HYPOMAGNESEMIA WITH MAGNESIUM DEPENDENT HYPOCALCEMIA

I A New Syndrome with Intestinal Magnesium Malabsorption

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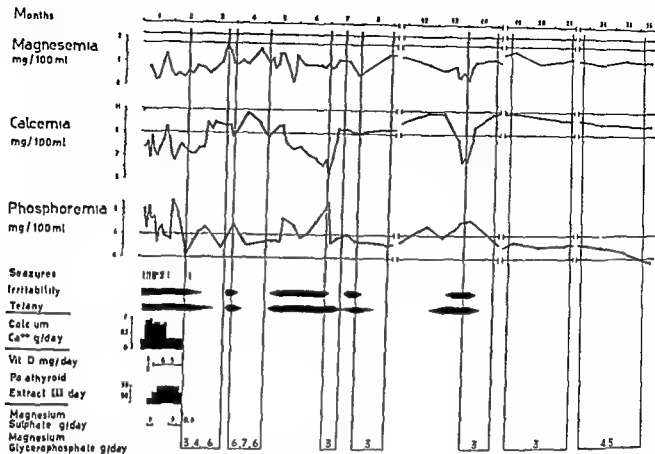


Fig 1 Clinical evolution and biochemical data of the case of primary hypomagnesemia on different treatments

METABOLIC INVESTIGATIONS

Methods Serum and urine Mg were determined by either the Mann & Yocis (21) or the atomic absorption spectrophotometric (Perkin & Elmer 303) (41) methods. The latter was used for Mg determination in washed feces, food, red cells, cerebrospinal fluid (c.s.f.) and sweat.

^{25}Mg total pool was determined according to Raynaud & Kellershohn (31) and Neer et al (26). ^{25}Mg intestinal absorption was determined by giving 20 μCi of this radioisotope by mouth without carrier after 12 hours fast and by measuring the radioactivity of the feces of 5 days.

The balance of stable Mg was performed in a metabolic ward according to the method of Rover (34). Body composition and exchangeable electrolytes were determined according to the method of Veall & Vetter (46).

Other investigations were performed by commonly accepted methods.

RESULTS

A low urinary elimination of Mg always corresponded to hypomagnesemia (Fig 2). Mg balance was variable but certainly reduced

and on one occasion negative. Intestinal absorption of both stable Mg and radioactive ^{25}Mg was also reduced (35) when compared to our control children on normal diet (Table 1).

Mg metabolism was also studied by i.v. injection of ^{25}Mg 19 days after discontinuing Mg therapy (plasma magnesium 1.1–1.2 mg/100 ml). Exchangeable pool accretion, urine and intestine excretion were clearly lower than in the case studied by Salet et al and in normal adults (35) and likely than in normal children of the same age (Table 2).

Mg concentration in sweat was increased while it was within normal range in red cells and c.s.f. (Table 1).

Intracellular K and K/Na ratio were low (Table 3).

With very low plasma magnesium the determination of some enzymes in serum (transaminase, cholinesterase, glutamic dehydro

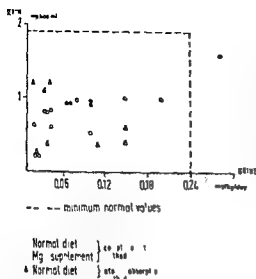


Fig. 2 Relationships between serum and urine Mg in the case of primary hypomagnesemia

enzyme) red cells (glucose-6-P-dehydrogenase) and muscle (creatin phosphokinase) gave normal results.

Glucose iv injection (0.03 mg/kg) provoked an increase of blood glucose (130°) and blood lactate (60°)

In the intestinal mucosa Mg-dependent ATPase histochemically studied (in the presence of Mg sulphate) appeared normal

Blood and urine electrolytes besides Mg, Ca and P were not significantly modified.

Table 1 Determinations of Vfg in the patient and in control subjects on normal Vfg diet or on low Vfg diet

	Mg balance				²⁵³ Mg Intestinal absorption (% 5 days)	CSF (mg/100 ml)	Sweat (mg/l)	Red cells (mg/g Hb)
	Intake (mg 5 days)	Output (mg 5 days) Stools Urine	Balance (mg 5 days)	Intestinal absorption (%)				
Patients	77.70	81.75 0.57	5.17	- 0.16				
on normal diet	77.70	~ 5.00 0.49	31.71	+ 18.83	+ 2.44	2.43	0.335	0.23
	77.70	195.70 0.75	+ 80.75	+ 29.13				
Mean values	77.70	34.15 0.60	+ 4.43	+ 15.93				
Control subjects								
on normal diet	857.40	781.50 69.95	+ 506.05	+ 67.17	+ 75.04 + 56.07	1.32-1.76	0.025-0.130	0.18
Control subject on low Mg diet					+ 60.39			

Table 2 *Mg metabolism of the patient with primary hypomagnesemia studied by ^{28}Mg i-v injection*

Exchangeable pool (mg/kg)	34.43
Accretion (mg/kg/day)	6.45
Urinary excretion (mg/kg/day)	0.019 (2.03%)
Intestinal excretion (mg/kg/day)	0.141 (2.59%)

Na Cl K Ca and P concentration in sweat was normal

Blood glucose plasma proteins lipides bilirubin and alpha amino nitrogen were normal as well as routine examination of c.s.f

No alteration of urinary 17 KS 17 KGS Porter Silber steroid was found Blood cortisol after ACTH stimulation was normal there were no signs of hyperaldosteronism

Fecal fat (one 5 days period) on normal diet was within normal range (0.6 g/day) xylose test (5 hours urinary elimination) in testinal mucosa (light microscopy) and intestinal X ray findings were normal.

An alteration of the apical part of the cytoplasm of epithelial cells of the intestinal mucosa was observed on electron microscopy dilated endoplasmic reticulum and mainly mitochondria swelling (Fig 3) The brush border was normal

Table 3 Na_e and K_e body content of the patient with primary hypomagnesemia

	Na_e (mEq/kg)	K_e (mEq/kg)
24 hour exchangeable pool	53.30 (norm 41.5)	21.80 (norm 40.0)
Extracellular	46.40	1.56
Residual (cell and bone Na_e)	7.90	
Intracellular		20.23

Renal function studies (creatinine clearance azotemia, urine examination, urine concentration power, aminoaciduria, glycosuria) gave normal results.

In the control boy fed with low Mg diet (Mg intake of 2.3 mg/kg/day), plasma magnesium and urinary magnesium dropped from 2 to 1.2 mg/100 ml and from 52 to less than 10 mg/day respectively. Intestinal absorption was clearly greater than in the patient with idiopathic hypomagnesemia (Table 1).

DISCUSSION

Nutritive requirement to maintain normal Mg homeostasis has been calculated to be between 4 and 6 mg/kg/day in adults but it is certainly higher in growing children (18-37). Nevertheless it is exceedingly high in our patient (about 30-70 mg/kg/day).

In spite of this high intake plasma Mg never

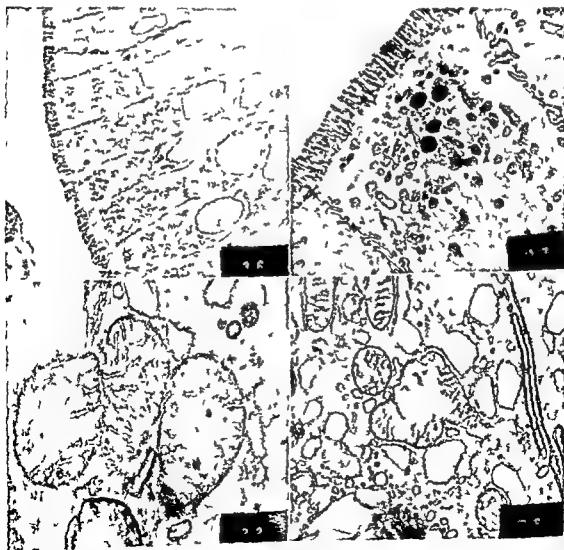


Fig. 3 Intestinal mucosa of the patient with primary hypomagnesemia (electron microscopy).

attains completely normal levels (Fig 1) when compared to normal values as determined by modern methods (4 5 21 43 45)

The exchangeable pool and the accretion of Mg are reduced more than in the case of Salet et al (35)

In the case of Paunier et al (29) both Mg and K were reduced in muscle. Because of a technical mistake we were not able to perform this determination but we found a diminished intracellular K and a low K_e/Na ratio (8 46). This agrees with the above mentioned results of Paunier et al (28) and with those obtained in Mg-deficient animals (23 47)

Mg-dependent ATPase was normal in the intestine but the histochemical reaction was performed *in vitro* by adding Mg sulphate. Therefore we cannot exclude an impairment of cation transport in chronic hypomagnesaemia as a consequence of an *in vivo* reduction of membrane ATPase regulating this transport secondary to Mg-depletion (20)

Erythrocyte Mg (Table 1) expressed in mg/l is similar to that of Salet's case (58 mg/l) and corresponds to normal values reported by other authors (4 38 44)

When we consider our own and the previously published cases (28 29) we can draw the following preliminary conclusions on this disease

1 hypomagnesaemia is associated to a body Mg-deficiency (exchangeable pool accretion muscle Mg)

2 Mg-deficiency is neither diffuse (normal c.s.f. and red cell Mg) nor so important that it determines an impairment of the Mg dependent metabolic activities (normal glycolysis)

3 there may be a secondary alteration of cation transport in tissues.

On the basis of these observations the hypothesis of either an increased excretion or a reduced absorption of Mg can be postulated.

Urinary magnesium directly depends on the supply and on the intestinal absorption of magnesium. With Mg-deficiency when renal

and endocrine functions are normal it diminishes to less than 12 mg/day in both adults (10 11 39) and children (our subject fed with low Mg diet). It is very low in our patient (Fig 2 and Table 3) and therefore it cannot explain hypomagnesaemia.

Intestinal secretion of Mg (1 35 40) was not increased (Table 3). Mg-concentration was increased in the sweat (Table 1) but this increase alone cannot adequately explain the Mg-deficiency in the patient.

Therefore the impairment of Mg absorption from the intestine seems to be the most probable explanation.

This hypothesis is also discussed by Friedman et al (12) and Paunier et al (29) but these authors studied only stable Mg balance. Salet et al (35) who determined stable Mg balance on Mg supplement and ^{25}Mg absorption (40 a) excluded the defect of the intestinal Mg transport.

In our case intestinal Mg malabsorption is clearly demonstrated by both stable Mg balance and oral ^{25}Mg administration (Table 1). Diarrhoea which as after experimental oral Mg load (7) frequently appeared further emphasizes this pathogenetic interpretation. It is particularly interesting to draw attention to the difference in the ^{25}Mg absorption in the patient and in the control boy fed with low Mg diet although considering the plasma magnesium (0.52 mg/100 ml and 1.2 mg/100 ml respectively) Mg requirement ought to have been greater in the former than in the latter (Table 1).

Paunier et al (29) speculate whether in children a poor utilization of Mg in the intestine is sufficient to produce a Mg-deficiency and a rapid fall of plasma magnesium. The results obtained in our control subject on low Mg diet allow us to answer Paunier's question positively.

Mg is chiefly absorbed in the small intestine (3 6 17 30). This absorption depends on the intestinal transport function (14 16) and is influenced by foods such as proteins (22) phytate phosphate fatty acids (2) vita

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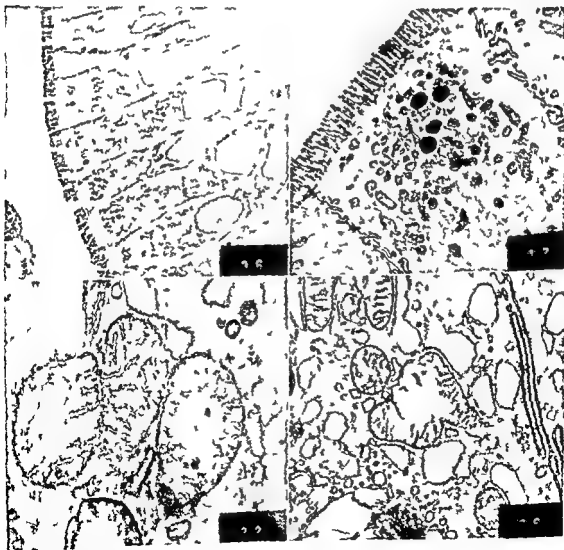


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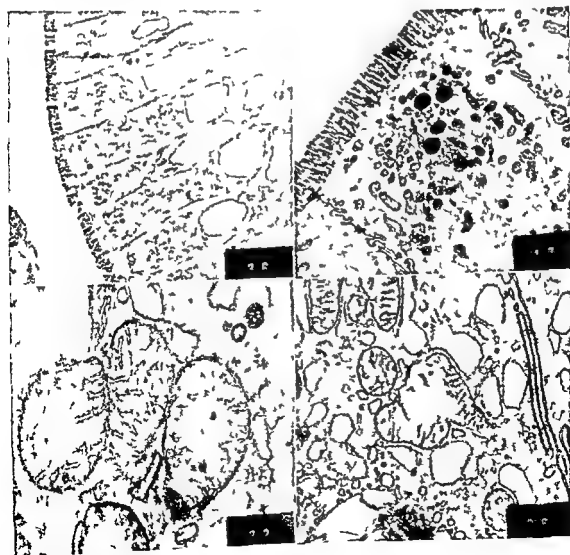


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min D (24-48) parathormone (19) and growth hormone (15)

In our patient no other manifestations of intestinal malabsorption were found. The diet was not responsible for the hypomagnesemia and the patient did not have rickets. Parathyroid function will be discussed in the next paper but there are no cases of hypoparathyroidism in which such a malabsorption has been demonstrated. No other endocrine disorders (hyperaldosteronism) are evident.

In conclusion 'chronic idiopathic hypomagnesemia is associated to a primary Mg-malabsorption'. This interpretation agrees with the opinion formulated by Seip¹ and more recently by Strømme et al (42).

For the present no definitive pathogenetic interpretation is possible for this new intestinal disease.

ATP ase transport system is not primitively impaired in the intestinal mucosa. The swelling of mitochondria of epithelial cells (Fig 3) is neither constant (42) nor a specific finding (27) in Mg malabsorption although a relationship between Ca and Mg transport, vitamin D and parathormone, mitochondria volume and function has been already emphasized on the basis of experimental investigations (9, 25, 27, 32-33).

Considering that the intestine is able to transport sufficient Mg only when its concentration in the intestinal lumen is unusually high, the hypothesis of an impairment of the simple diffusion of Mg seems to be rather acceptable (42).

CONCLUSIONS

The new syndrome of chronic hypomagnesemia with Mg dependent hypocalcemia is characterized by primary Mg malabsorption. An alteration of the simple diffusion of Mg seems likely. It may be associated with mitochondria swelling.

¹ Our results concerning Mg malabsorption were presented at the Symposium on Inborn Errors of Metabolism (Zurich June 1968) on the discussion of Seip's communication.

Taking into consideration the high concentration of Mg in sweat, we demonstrated for the first time a more extensive impairment of Mg transport cannot be excluded in this condition.

Decreased urinary excretion of magnesium was present but we did not study the renal clearance of this cation. Therefore we cannot establish the actual tubular reabsorptive function in this disease. Considering possible functional relationships between intestinal mucosa and tubule we think it will be interesting to study the transport of Mg in other cases of Mg malabsorption.

SUMMARY

Description of a case of chronic hypomagnesemia with secondary hypocalcemia, tetany and convulsions followed for a period of 3 years from the age of 7 months. Primary malabsorption of magnesium has been demonstrated. Mitochondria of intestinal mucosa cells were found to be swollen. Mg-concentration was increased in sweat.

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CONCLUSIONS

The new syndrome of chronic hypomagnesemia with Mg dependent hypocalcemia is characterized by: primary Mg malabsorption. An alteration of the simple diffusion of Mg seems likely. It may be associated with mitochondria swelling.

Our results concerning Mg malabsorption were presented at the Symposium on Inborn Errors of Metabolism (Zurich June 1968) on the discussion of Seip's communication.

Taking into consideration the high concentration of Mg in sweat we demonstrated for the first time a more extensive impairment of Mg transport cannot be excluded in this condition.

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II A Study of the Relationship between Magnesium Calcium and Strontium

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the Department of Paediatrics University of Berne Berne Switzerland and the Department
of Child Health Paediatric Institute Burlo Garofalo University of Trieste Trieste Italy

In a previous paper (23) we presented a case of idiopathic hypomagnesemia with secondary hypocalcemia hyperphosphatemia. Only a very low intake of Mg normalized clinical picture and the plasma calcium and phosphorus. A primary intestinal malabsorption of Mg was demonstrated.

In this paper we shall attempt to evaluate the complex relationship between Mg, Ca and P on the basis of investigations performed in the case of idiopathic hypomagnesemia, a control boy fed with a low Mg diet and a group of control children who received a Mg supplement in the diet.

METHODS

Calcium balance of stable Ca was determined according to Royer (30).

Sr and ^{45}Ca intestinal absorption was determined respectively giving 10 μCi and 3 μCi of these isotopes by mouth without carrier after a 12 hr fast and by measuring the radioactivity of the urine over 5 days. Both isotopes are separated by gamma spectrometry.

Calcium total pool was determined according to Ray and Kellershohn (28) and Neer et al (22). Human determinations were performed by commonly acceptable methods.

Parathyroid extract (Lilly) activity was established by studying renal clearance of P in control children.

This investigation was supported by grant 4580 from the National Foundation for Scientific Research and National Suisse de la Recherche Scientifique.

The low Mg diet administered to a control boy contained 23 mg/kg/day. Ca content of the diet was normal.

Other control children were given a normal diet containing a supplement of 5 g/m/day of Mg glycerophosphate.

RESULTS

Ca, P and Sr metabolism in idiopathic hypomagnesemia

The dependence of normal calcium and phosphorus blood levels on Mg supplements and the resistance to parathyroid extract and vitamin D treatment were demonstrated in our patient (Fig 1 of the previous paper).

Hypocalcemia was always associated with low urinary calcium values (0.09–0.7 mg/kg/day). On more or less prolonged Mg therapy with plasma calcium between 7 and 10 mg/100 ml, urinary calcium fluctuated between 0.3 and 2.2 mg/kg/day.

Calcium balance was positive and calcium intestinal absorption was only a little lower than in control subject studied under the same conditions (Table 1). ^{87}Sr intestinal absorption was on three occasions practically zero, either on normal diet or on Mg supplement (Table 1). The ^{45}Ca iv injection performed after Mg therapy was discontinued and when plasma calcium was very low (6.6 mg/100 ml) and plasma phosphorus increased (7.1 mg/100 ml).

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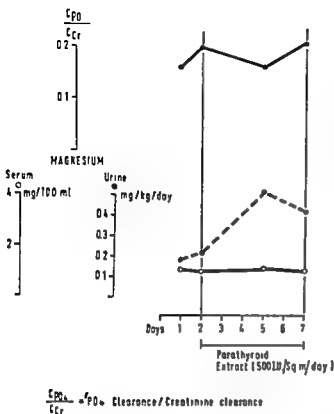


Fig 1 Ellsworth Howard test in patient with primary hypomagnesemia

demonstrated a reduced exchangeable pool low urinary excretion normal values of accretion and no intestinal hypersecretion of Ca (Table 2)

On normal diet with plasma phosphorus

Table 2 Calcium metabolism of the patient with primary hypomagnesemia studied by ^{45}Ca i-v injection

	Patient	Normal children
Exchangeable pool (mg/kg)	75.5	139 ± 46
Accretion (mg/kg/day)	53.7	46.7 ± 12.2
Urinary elimination (mg/kg/day)	0.3	4 ± 2
Intestinal elimination (mg/kg/day)	3.2	3.18 ± 1.06

between 5.2 and 7.1 mg/100 ml urinary phosphorus fluctuated between 10.7 and 36 mg/kg/day and phosphorus renal clearance was not significantly modified (tubular reabsorption 81–91%)

On Mg therapy the normalization of plasma phosphorus corresponded to a lowering of the urinary phosphorus (5.1–8.3 mg/kg/day)

The injection of parathyroid extract (Ellsworth Howard test) performed 25 days after Mg therapy was discontinued (plasma calcium 10.6 mg/100 ml plasma phosphorus 7 mg/100 ml plasma magnesium 0.32 mg/100 ml) did not provoke any modification of the phosphorus renal clearance/creatinine renal clearance ratio (Fig 1) Blood Mg level was unmodified while urinary magnesium was increased by parathyroid extract (Fig 1)

Table 1 Ca balance and ^{85}Sr absorption in a patient with primary hypomagnesemia and control subjects

	Ca ⁺⁺ -balance				^{85}Sr Intestinal absorption (5 days) (n v 43–88)
	Intake (mg/5 days)	Output (mg/5 days)		Balance (mg/5 days)	
		Stools	Urine		
Patient on normal diet	2 677.50	1 281.00	25.25	+1 371.25	+52.15
	2 677.50	1 222.50	20.98	+1 434.02	+54.34
	2 677.50	1 396.50	22.75	+1 258.25	+47.84
Mean value	2 677.50	1 300.00	22.99	+1 354.5	+51.44
Patient on Mg supplementation					0.00
Control subjects on normal diet	2 655.00	985.85	85.15	+1 584.00	+62.87
Control subject on experimental ^a Mg deficiency					52.4 ± 18
					46.29

^a 47 days of low Mg diet

Howard test was also performed. In the schema (Fig 2) columns indicate the 24-hours urinary excretion of P and Ca, the dotted line indicates the plasma calcium values before and after 3 hours of calcium perfusion and the continuous line indicates the 24-hours phosphorus renal clearance before and after calcium perfusion.

Without Mg therapy (plasma calcium 6.5 mg/100 ml, plasma phosphorus 7 mg/100 ml, plasma magnesium 0.32 mg/100 ml) no significant modifications of renal output of calcium and phosphorus were observed. Plasma calcium increased and Ca retention is practically 100%. On Mg therapy the retention of phosphorus renal clearance was very evident and the high Ca retention persisted (98%).

X-ray findings did not demonstrate significant evidence of bone mineralisation (fe-

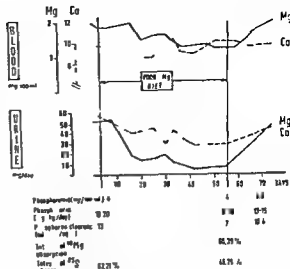


Fig 3 Modification of biochemical data in a control child on a low Mg diet.

mur and tibia metaphysis are a little thickened). Serum alkaline phosphatase was always within normal range (35–89 i.u.).

Ca, P, Sr and Mg metabolism in control children fed with either low Mg diet or rich Mg diet

In a boy fed with a prolonged low Mg diet with normal Ca supply, urinary magnesium fell quickly. Plasma magnesium also fell but slower and to a lesser degree. Intestinal absorption of ^{45}Mg was raised. It was much higher than in the patient with primitive hypomagnesemia (Fig 3, Table 1 of the previous paper).

Plasma calcium was practically unchanged but urinary calcium fell. ^{86}Sr intestinal absorption also fell but it did not attain the low values found in the patient with primary chronic hypomagnesemia (Table 1). Urine phosphorus excretion and phosphorus renal clearance fell without any significant modification of plasma phosphorus (Fig 3).

In this boy Howard test was also performed on normal diet and on low Mg diet. On a low Mg diet the P renal clearance diminished slightly and the Ca body retention was high. When a normal diet was given again for 15

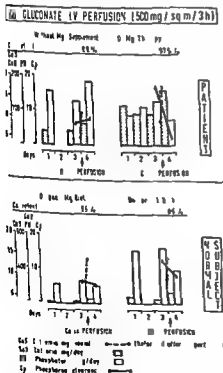


Fig 2 Howard test in patient with hypomagnesemia, on a normal Mg intake then and on Mg supplements and in a control subject, on low Mg intake and then on normal diet.

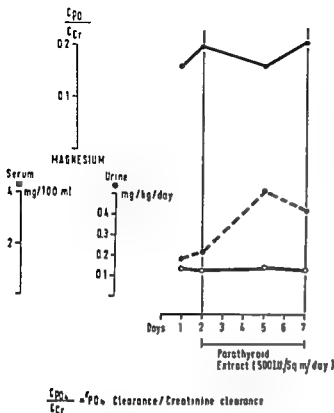


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between 5.2 and 7.1 mg/100 ml urinary phosphorus fluctuated between 10.7 and 36 mg/kg/day and phosphorus renal clearance was not significantly modified (tubular reabsorption 81–91%)

On Mg therapy the normalization of plasma phosphorus corresponded to a lowering of the urinary phosphorus (5.1–8.3 mg/kg/day)

The injection of parathyroid extract (Ellsworth Howard test) performed 25 days after Mg therapy was discontinued (plasma calcium 10.6 mg/100 ml, plasma phosphorus 7 mg/100 ml, plasma magnesium 0.32 mg/100 ml) did not provoke any modification of the phosphorus renal clearance/creatinine renal clearance ratio (Fig 1). Blood Mg level was unmodified while urinary magnesium was increased by parathyroid extract (Fig 1).

Table 1 Ca balance and ^{85}Sr absorption in a patient with primary hypomagnesemia and control subjects

	Ca ⁺⁺ -balance			^{85}Sr Intestinal absorption	
	Intake (mg/5 days)	Output (mg/5 days) Stools Urine	Balance (mg/5 days)	Intestinal absorption ()	(n v 43–88)
Patient on normal diet	2 677.50	1 281.00 25.25	+ 1 371.25	+ 52.15	
	2 677.50	1 222.50 20.98	+ 1 434.02	+ 54.34	0.32
	2 677.50	1 396.50 22.75	+ 1 258.25	+ 47.84	0.00
Mean value	2 677.50	1 300.00 22.99	+ 1 354.5	+ 51.44	0.16
Patient on Mg supplementation					0.00
Control subjects on normal diet	2 655.00	985.85 85.15	+ 1 584.00	+ 62.87	52.4 ± 18
Control subject on experimental ^a Mg deficiency					46.29

^a 47 days of low Mg diet

high) and the Mg glycerophosphate supply favours the absorption of both ^{45}Ca and ^{87}Sr but chiefly of the latter (Figs 3 and 4)

Of course there are many data which emphasize that more than one transport mechanism exists for these cations. It seems that Mg , and Sr are linked to each other more than Mg and Ca in intestinal transport level

The influence of Mg on Ca P homeostasis

The interpretation of this influence is particularly difficult. Both in primary chronic hypomagnesemia and experimental human (8, 15 personal observation Fig 3), puppy, baby pig and calf (15, 16, 20) Mg deficiency plasma calcium and/or urinary calcium levels are low. In our case of primary Mg malabsorption and in Salet's one (31) this lowering is associated to a reduced exchangeable pool of Ca . In both cases no hypersecretion of Ca into the intestine is demonstrated and the malabsorption of exogenous Ca is not so much reduced as to explain such a severe alteration of Ca homeostasis.

In our own and in three other cases (8, 31, 36) there is also high plasma phosphorus but according to other authors (9, 16, 19, 25, 27, 31) three arguments are against primitive hypoparathyroidism:

1 although a Mg-deficiency is demonstrated by Gill et al (10) in hypoparathyroidism, magnesium is always exceedingly low.

2 parathyroid extract does not correct hypomagnesemia.

3 Mg therapy completely cures hypoparathyroid like symptoms. Moreover phosphorus renal clearance is not clearly reduced.

Secondary hypoparathyroidism is also unlikely since in calves (16) and sheep (3) an inhibition instead of an activation of parathyroid function by Mg has been stressed.

On the contrary our patient shows a hyposensitivity to exogenous parathyroid extract and vitamin D (first phase of the course of the disease and Ellsworth Howard test) and to the fluctuation of endogenous parathormone induced by the Ca perfusion (Howard

test) (Figs 1 and 2 and Fig 1 of the previous paper). Such a hyposensitivity was also demonstrated in the case of Paunier et al (25) in human hypoparathyroidism with secondary Mg deficiency (18) and in Mg-deficient hypocalcemic calves (16). Moreover the role of Mg in regulating the sensitivity to parathormone may be confirmed by the results obtained with the Ellsworth Howard test in the boy fed with a low Mg diet (Fig 2).

On the contrary in the case of primary hypomagnesemia of Strømme et al (36) the increase of plasma calcium after parathyroid extract treatment was greater than in control subjects.

In our case increased urinary magnesium without any significant modification of plasma magnesium on parathyroid extract treatment (Fig 1) may be interpreted as a consequence of a mobilisation of residual Mg from bones and contrasts with the Mg tubular reabsorption stimulating function of parathormone (19).

Normal Ca accretion in our case and in Salet's one contrasts with the reduced exchangeable Ca pool (Table 1) but is compatible with good bone mineralisation demonstrated by both Howard test and X ray findings.

In hypocalcemic Mg-deficient calves Larvor et al (16) demonstrated a low mineral dynamic. Considering that pyrophosphatase which is certainly involved in the regulation of bone mineral dynamic is Mg-dependent and the fact that Mg increases Ca removal from bone in parathyroidectomized animals and cures experimental hypoparathyroidism (4, 6) an impairment of local factors regulating bone mineral turnover cannot even be excluded.

Variable interrelations may exist between the degree of intestinal Mg and Ca absorption, parathormone production, parathormone and vitamin D sensitivity and the Mg-dependent bone factors regulating mineral dynamic in the different cases of chronic idiopathic hypomagnesemia with secondary hypocalcemia, hyperphosphoremia.

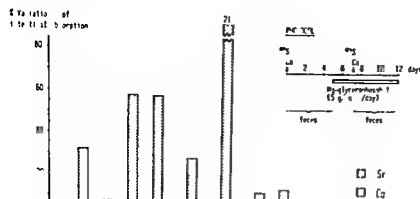


Fig 4 The influence of oral Mg glycerophosphate on ^{86}Sr and ^{45}Ca intestinal absorption

days (plasma magnesium 1.8 mg/100 ml) the reduction of the P clearance was more evident and the retention of Ca on Ca intra venous perfusion diminished a little (Fig 2)

In a few other control children the supplementation of the diet with high dose of Mg glycerophosphate provoked a more or less evident increase of the intestinal absorption of ^{86}Sr ^{45}Ca intestinal absorption was also increased but this effect of the Mg supplement was less marked and occurred only in 2 of the 5 studied children (Fig 4)

DISCUSSION

The investigation performed in the patient raises the problem of the relationship between Mg, Ca and Sr in intestine and the influence of the alteration of Mg metabolism on Ca P homeostasis

The research carried out on control subjects allows us a better interpretation of this problem

Therefore all the results we obtained will be discussed conjointly

The relationship between Mg, Ca and Sr absorption

In the patient with Mg malabsorption Ca balance is more positive than Mg balance as in other similar cases (9, 25, 31) and although balance data must be interpreted with caution, intestinal Ca absorption cannot be considered as certainly reduced (Table 1). On the contrary a very low ^{86}Sr absorption exists (Table 1). Of course in this case the relation

ship between Mg, Ca and Sr at intestinal transport level seems to be rather complex

Many authors (1, 14, 21, 24, 33, 34) stress the competition between Ca, Mg and Sr for a common transport pathway. Ogata et al (24) postulate that factors such as parathormone, which activate Ca transport, primitively act on Mg transport. Hendrix et al (14) emphasize on the contrary that besides a common transport pathway there are specific mechanisms for the transport of these cations. Laster & Ingelfinger (17), Ross (29) and Strömme et al (36) postulate that a saturating active process or a facilitated diffusion are involved in the transport of Mg. Rasmussen & Ogata (26) stress that Mg and Ca transport depends on an unique mitochondrial source of energy but that two carriers are involved in this transport. Heaton et al (13) emphasize a common pathway for Ca and Mg absorption but they state that vitamin D acts more on the former than on the latter. Clark (5) demonstrated that Mg supply increases or reduces intestinal Ca absorption according to the Ca content of the diet. Moreover although it is generally admitted that Sr transport parallels the Ca one, a few observations show that differences exist in discrimination and transport speed of these cations in intestine and kidney (5, 7, 11, 12, 14, 32).

The complex relationship between Mg, Ca and Sr at intestinal level are also demonstrated by the research we performed in normal children. A prolonged low Mg diet reduces the ^{86}Sr intestinal absorption (^{86}Sr absorption is

high) and the Mg glycerophosphate supply favours the absorption of both ^{45}Ca and ^{87}Sr but chiefly of the latter (Figs 3 and 4)

Of course there are many data which emphasize that more than one transport mechanism exists for these cations. It seems that Mg and Sr are linked to each other more than Mg and Ca at intestinal transport level.

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Variable interrelations may exist between the degree of intestinal Mg and Ca absorption, parathormone production, parathormone and vitamin D sensitivity and the Mg-dependent bone factors regulating mineral dynamic in the different cases of "chronic idiopathic hypomagnesemia with secondary hypocalcemia hyperphosphoremia".

CONCLUSIONS

Magnesium in the diet plays an important role in regulating Ca and Sr absorption

Probably a part only of the mechanism regulating the absorption of these cations is impaired in primary hypomagnesaemia and one cannot exclude that it mainly regulates Mg and Sr passive transport

Mg deficiency secondary to Mg malabsorption, provokes an alteration of Ca P homeostasis. Parathormone and vitamin D resistance is clear enough but the whole pathogenesis of the alteration of Ca P homeostasis is unknown and further investigation is necessary to understand it better

SUMMARY

A study has been carried out on the relation ship between Mg, Ca and Sr in a case of primary Mg malabsorption and in control children on high and low Mg diets

Probably more than one transport system exists for these cations in intestine and in Mg-malabsorption the simple diffusion of both Mg and Sr is impaired

The influence of Mg on Ca P homeostasis is not clearly understood but a resistance to parathormone and vitamin D in Mg deficiency is emphasized

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PIMARICIN (NATAMYCIN) IN THE TREATMENT OF SUPERFICIAL FUNGAL INFECTIONS IN CHILDREN

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Among the relatively few antifungal antibiotics which have found practical application in medical treatment, pimaricin (natamycin) is prominent because of its wide spectrum of activity against not only yeast-like fungi, but also moulds and dermatophytes and *Trichomonas vaginalis* (1, 7 10, 18, 21, 22) Discovered in 1955 by Struyk and coworkers (27), it is produced by *Streptomyces natalensis* Its tetraen structure is similar to but not identical with nystatin (20) In view of its wide spectrum of activity it aroused the interest of clinicians and was applied first of all in dermatology and gynecology (2-6, 11-14 16 19, 23, 25, 26 28,29)

Since the absorption of pimaricin in the intestinal tract is poor, it is used mainly locally Oral tablets may be used complementary to local treatment Pimaricin is available in various preparations suitable for the treatment of different fungal infections

PROCEDURE

This report concerns clinical studies of four preparations of pimaricin¹ pimafucin suspension 1 pimafucin vaginal tablets pimafucin ointment and pimafucort ointment The patients considered in the study were treated in the Paediatric Institute Medical Academy in Kraków They were in part hospitalized and in part treated as out patients A total of 69 children and young people up to age eighteen were treated Among them there were 59 children up to age fourteen and ten from age fourteen to eighteen

¹ Mycofarm Delft Holland

Twenty four patients with vaginitis were treated with pimafucin vaginal tablets Among them there were 15 girls aged eight to fourteen and 9 older girls aged fifteen to eighteen years Sixteen patients were previously treated without success with various antifungal preparations and antiseptics including nystatin marcostatin borax with glycerin gentian violet and sterovag One patient was previously treated with metranidazole and one with sigmamycin In this group of patients symptoms persisted for a long time ranging from 3 months to 7 years Nystatin was previously applied without success in 9 of these cases In 8 patients previously untreated symptoms appeared from 3 weeks to 14 months earlier The applied treatment with pimafucin vaginal tablets was local and included one or two courses each lasting 20 days Every night before retiring one tablet cut along into two pieces was introduced into vagina by means of narrow forceps Disappearance of clinical symptoms and negative results of three mycological examinations performed after 1 4 and 8 weeks after discontinuation of the therapy were considered as complete cure Cases of vulvovaginitis in which complete cure was not achieved were submitted to the second course of treatment

Fourteen children aged from 2 days to 12 years suffering from oral thrush or stomatitis with secondary yeast like fungi infections were treated with pimafucin suspension 1 Four to five drops of the drug were given under the tongue or to the lesion itself in small children and seven to ten drops in older children

Thirty one children with different skin infections as angulus infectiosus intertrigo otitis externa candidiasis unguum were treated with pimafucin or pimafucort ointments The treatment consisted of direct application of ointment lightly rubbed in twice or several times daily sometimes under dressing during 5 to 105 days

Among patients treated with pimafucin suspension 1 pimafucin or pimafucort ointments the symptoms before the treatment persisted for different lengths of time from several days in the cases of oral thrush

Table 1 Results of treatment with different pimaricin preparations in children

Diagnosis	Total number examined	Pimaricin preparation applied	Results of treatment		
			Cure	Improvement	No success
Vulvovaginitis	24	Pimaricin vaginal tablets	20	3	1 ^a
Candidias mucosae oris	8	Pimaricin suspension 1	4	1	3 ^b
Oral aphthosa (with secondary yeast like fungi infection)	3	Pimaricin suspension 1 ^a	3	—	—
Oral ulcerosa (with secondary yeast like fungi infection)	3	Pimaricin suspension 1	1	2	—
Oral infectious oris	3	Pimaricin ointment	2	1	—
Oral blastomycetia	1	Pimaricin ointment	1	—	—
Oral ringo regionis	3	Pimaricin ointment	2	1	—
Oral blastomycetia	3	Pimaricin ointment	2	1	—
Oral candidias unguum	3	Pimaricin ointment	2	1	—
Oral infectious oris	4	Pimaricort ointment	3	1	—
Oral externa chronica	3	Pimaricort ointment	3	—	—
Oral ringo regionis	11	Pimaricort ointment	11	—	—
Oral blastomycetia	3	Pimaricort ointment	1	2	—
Oral candidias unguum	3	Pimaricort ointment	1	2	—
Total	69		53 76.8	12 17.3	4 5.7

^a Concerns the case of diabetes mellitus.

^b Concerns two cases of leukemia.

14 months in the cases of intertrigo and otitis externa. When the clinical symptoms had lasted for a short time no previous treatment was applied. In other cases there were used without success such medicaments as gentian violet borax with glycena and antiseptic ointments. Whenever clinical samples could be taken the diagnosis was confirmed on the basis of mycological examination. It consisted of direct microscopic examination and culture on Sabouraud glucose agar slopes. The isolated fungi were identified according to commonly accepted morphological and biochemical characteristics (9, 15). The cure was evaluated on the basis of clinical picture and negative results of culture performed one to three times after 4 to 30 days from the time the therapy was ended.

RESULTS

The results of treatment of different mycotic infections by means of four studied pimaricin

preparations are presented in the table. In the cases of vulvovaginitis *Candida albicans* was found in 13 patients, other species of *Candida* (*C. krusei*, *C. parakrusei*, *C. tropicalis*, *C. pseudotropicalis*) in 6. *Torulopsis* sp. in 2. Double infection with *Candida albicans* and *Geotrichum* sp. was shown in one patient and in two there was double infection with moulds (*Penicillium* sp. and *Scopulariopsis* sp.). In 45 other cases of different fungal infections *Candida albicans* was found in 32, *Candida krusei* in one, *Rhodotorula* sp. in 2 and *Aspergillus fumigatus* in 2. Mixed infections with different species of *Candida* and *Geotrichum* sp. (*C. albicans* + *C. Tropicalis*, *C. albicans* + *C. parakrusei*, *C. pseudotropicalis* + *Geotrichum* sp., *C. albicans* + *C. pseudotropicalis* + *Geotrichum*

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Among the relatively few antifungal antibiotics which have found practical application in medical treatment, pimarin (natamycin) is prominent because of its wide spectrum of activity against not only yeast like fungi, but also moulds and dermatophytes and *Trichomonas vaginalis* (1, 7, 10, 18, 21, 22) Discovered in 1955 by Struyk and coworkers (27) it is produced by *Streptomyces natalensis* Its tetraen structure is similar to but not identical with nystatin (20) In view of its wide spectrum of activity it aroused the interest of clinicians and was applied first of all in dermatology and gynecology (2-6 11-14, 16, 19 23, 25 26 28 29)

Since the absorption of pimarin in the intestinal tract is poor it is used mainly locally Oral tablets may be used complementary to local treatment Pimarin is available in various preparations suitable for the treatment of different fungal infections

PROCEDURE

This report concerns clinical studies of four preparations of pimarin: pimafucin suspension 1% pimafucin vaginal tablets pimafucin ointment and pimafucort ointment The patients considered in the study were treated in the Paediatric Institute Medical Academy in Kraków They were in part hospitalized and in part treated as outpatients A total of 69 children and young people up to age eighteen were treated Among them there were 59 children up to age four and ten from age fourteen to eighteen

¹ Mycofarm Delft Holland

Twenty four patients with vaginitis were treated with pimafucin vaginal tablets Among them there were 15 girls aged eight to fourteen and 9 older girls aged fifteen to eighteen years Sixteen patients were previously treated without success with various antifungal preparations and antiseptics including nystatin miconazole borax with glycerin gentian violet and sterovag One patient was previously treated with metranidazole and one with sigmamycin In this group of patients symptoms persisted for a long time ranging from 3 months to 7 years Nystatin was previously applied without success in 9 of these cases In 8 patients previously untreated symptoms appeared from 3 weeks to 14 months earlier The applied treatment with pimafucin vaginal tablets was local and included one or two courses each lasting 20 days Every night before retiring one tablet cut along into two pieces was introduced into vagina by means of narrow forceps Disappearance of clinical symptoms and negative results of three mycological examinations performed after 1 4 and 8 weeks after discontinuation of the therapy were considered as complete cure Cases of vulvovaginitis in which complete cure was not achieved were submitted to the second course of treatment

Fourteen children aged from 2 days to 12 years suffering from oral thrush or stomatitis with secondary yeast like fungi infections were treated with pimafucin suspension 1% Four to five drops of the drug were given under the tongue or to the lesion itself in small children and seven to ten drops in older children

Thirty one children with different skin infections as angulus infectiosus intertrigo otitis externa candidiasis unguis were treated with pimafucin or pimafucort ointments The treatment consisted of direct application of ointment lightly rubbed in twice or several times daily sometimes under dressing during 5 to 105 days

Among patients treated with pimafucin suspension 1% pimafucin or pimafucort ointments the symptoms before the treatment persisted for different lengths of time from several days in the cases of oral thrush

lous. Complete cure was achieved after only one course of treatment with pimafucin vaginal tablets. Excellent results were obtained in the treatment of otitis externa chronica which was also observed by Alteras (1). In these cases the lesions were healed within 10-14 days by the application of pimafucort ointment.

SUMMARY

Sixty nine children suffering from superficial fungal infections were treated with four pimarin preparations: pimafucin vaginal tablets, pimafucin 1° suspension, pimafucin and pimafucort ointments. Complete cure was achieved in fifty three children (76.8%) improvement in twelve (17.3%). In four (5.7%) children with serious basic diseases (leukaemia, diabetes) the treatment was unsuccessful. No signs of intolerance were observed.

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sp) were detected in 5 patients. In 3 cases mycological examinations were not performed.

Among the cases of vulvovaginitis in 16 patients full clinical cure with three negative cultures was achieved after one course of treatment. Eight other patients were submitted to the second course of treatment and cure was achieved this time in 4 cases, with improvement in 3 other cases. In one case only the treatment failed even after the third course. It should be pointed out that it was a case of poorly controlled juvenile diabetes mellitus. Two other girls who improved after the second course of treatment suffered from diabetes mellitus too. Summarizing all the cases of vulvovaginitis treated with pimafucin vaginal tablets, cure after one or two courses was achieved in 20 girls and improvement in 3.

Pimafucin suspension 1% applied for the treatment of lesions localized in mouth resulted in complete cure of 8 patients, there was improvement in 3 cases and in 3 others the treatment was unsuccessful. In the latter group, 2 children were suffering from leukemia and were in poor general condition.

After the therapy with pimafucin or pimafucort ointments applied in 31 children cure was achieved in 25 cases and improvement in 6.

Considering all the results of treatment with different pimafucin preparations totalling 69 children, cure was noted in 53 (76.8%) and improvement in twelve (17.3%). In four (5.7%) children mainly with serious diseases treatment was unsuccessful. In no case was any sign of intolerance observed.

DISCUSSION

Recently there have appeared many reports on advantageous curative effects of pimafucin in fungal infections of skin and mucous membranes (9, 23). The effectiveness of the new antibiotic was emphasized especially in infections caused by yeast like fungi of *Candida* species. The latter are wide spread in nature and have an important position in human pathology, particularly *Candida albicans* which

is closely linked with living human or animal organisms (8). It may be present in normal mucous membranes of the mouth and vagina, hence the first contact with this microorganism in newborn children occurs in the genital tract of the mother. An excessive multiplication of microorganisms and in consequence development of illness takes place in the conditions of resistance deficiency such as blood diseases, metabolic disturbances, or during prolonged therapy with antibacterial broad spectrum antibiotics or corticosteroids. *Candida* infections often occur in children and are dangerous especially for infants whose mechanisms of natural resistance are not fully developed (29). Hence in this report, dealing with children *Candida* infections dominated and were observed in the form of stomatitis, oral thrush, angulus infectiosus and intertrigo. Vulvovaginitis caused by yeast like fungi is not rare in girls during puberty and may present a serious therapeutical problem.

Not many papers published so far have dealt with the application of pimafucin in children. In this respect the report of Van Den Driessche & Bottu (29) is noteworthy. The authors treated successfully with pimafucin skin and intestinal tract candidiasis in small children. Much attention was paid to the treatment of vulvovaginitis of mycotic or protozoal etiology (4, 6, 12, 13, 17, 19, 22-24), and further attention was given to the problem of treatment of vulvovaginitis in pregnant women where the elimination of *Candida* infection is of particular importance in view of possible infection of the newborn child (35). The results of our attemptive treatment of vulvovaginitis in girls correspond to those obtained by other authors in the treatment of adult women and assured satisfactory percentage of healed cases. A noteworthy case of a 17 year old girl suffering from persistent vulvovaginitis since the age of ten was observed. She had been treated unsuccessfully with nystatin, borax with glycerin, gentian violet and sterovag. Before starting that treatment with pimafucin *Candida pseudotropicalis* was cultured from her vaginal secre-

DIETARY COMPOSITION AND DENTAL DISEASE IN ADOLESCENT DIABETICS

A pilot study

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The relationship between diabetes mellitus and dental health has long been a matter of discussion. The current opinion seems to be that diabetes has a modifying effect on the incidence of dental caries (8) and an accelerating effect on locally initiated periodontal disease (4). There is suggestive evidence that dietary habits play an important role in the pathogenesis of dental caries (2, 13) and in the development of supragingival calculus (4, 5, 9).

Two cross sectional surveys conducted with a five year interval of dietary intakes among adolescent diabetics (10, 12) revealed that they consumed a higher protein and lower carbohydrate percentage of total calories than nondiabetic controls. Those factors favouring dental health among nondiabetics (2, 15) might be counteracted by the prevalence of continuously elevated blood glucose and probably salivary glucose concentration in most juvenile diabetics (4, 11). It thus seemed of interest to compare directly the dietary composition with the result of a dental examination in groups of diabetics and nondiabetics.

MATERIAL AND METHODS

The experimental groups were part of a larger population under continuous follow up in a longitudinal study (11, 1). The material consisted of 43 subjects, 24 diabetics and 17 nondiabetics who attended the follow up examination during a determined period of 3 weeks.

They were below 25 years and were living in the city of Stockholm. None of the subjects had knowledge in advance about the dental examination to be performed.

The dietary intakes were assessed by the 24-hour recall method. The interviews were planned conducted and the dietary composition calculated as previously described (10). Each subject was interviewed twice with 10-20 days intervals; the second recall at the day of dental examination. During the week before the dental examination all subjects also kept a dietary record.

Dental caries was quantitated in each case by the 100-surface index (14) and evaluated according to an established method using simultaneous technique (6). A similar technique was used in the registration of dental calculus of supragingival type (4). Oral hygiene was recorded at the second interview as the number of tooth brushings per day.

The degree of diabetic control was evaluated in each patient as previously described (11) using mean degree of glycosuria over 24 hours and fasting blood glucose values at outpatient visits over a period of 5 years.

RESULTS

The diabetics had a mean age of 17.4 years (range 13-19 yr) and the nondiabetics 17.3 years (range 15-23 yr). The heights and weights for each subject fell within ± 1 SD for normals as did the weight/height ratio. The mean age at onset of diabetes was 7.5 years (range 2-13 yr) and the mean duration 9.8 years (range 5-18 yr). The controls were chosen according to the "social twin" principle.

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significant correlations were observed. The duration of diabetes was not obviously related to dental caries.

COMMENTS

The general pattern of lower intake of refined carbohydrates taken less frequently among the diabetics than among the nondiabetics could well play a part in explaining the tendency to a lower frequency of dental caries among diabetics.

(1 2 3) A minor significance would be given to elevated blood glucose levels. The formation of calculus is due to calcification of dental plaque material. Sucrose is an excellent substrate for some acid producing microorganisms also essential for the plaque building process (1 7). Since the diabetics consumed relatively little refined carbohydrates, mainly sucrose, they would have a lower frequency of supragingival calculus than the nondiabetics. However, the higher protein content of the diabetic diet might increase the buffer capacity in mixed saliva, i.e. the capacity to interact pH-changes. A high buffering capacity has been suggested to favour calculus formation among nondiabetics (2 5 9 15) and might thus explain the somewhat higher frequency of calculus among the diabetics in this material.

A true picture of the degree of diabetic control over a long period of many years will probably never be obtained (11). Recognising that the available parameters are at best only estimates of diabetic control, it is not surprising that in the present small material no correlation to dental health was found.

The 24 hour recall method had good reproducibility (Table 1) both in the diabetic and the nondiabetic group. On the other hand there was a considerable decrease in the subjects' own reported caloric intake in comparison with the results of interviews, consistent with reports in normal individuals (16). The most obvious drop was found in the consumption of refined carbohydrates among the diabetics, suggesting that self recording methods should not be used

for nutritional surveys in subjects who are prescribed specific diets.

SUMMARY

In a group of adolescent diabetics there was a probable lower frequency of caries but a higher frequency of supragingival calculus than in the control group. The diabetics consumed relatively more protein and less refined carbohydrates taken less frequently than nondiabetics. The 24 hour recalls showed good reproducibility but an unsatisfactory correspondence to a 7 day record among diabetics.

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Table 1 Comparison between mean dietary intakes at two "24 hour recalls" (I and II) and one 7-day record (III)

No of cases	Calories			Protein (g and %)			Fat (g and %)			Carbohydrate (g and %)			Refined carbohydrate (% of total)		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
<i>Diabetics</i>															
15 boys	2 782	2 705	2 303	93.8	98.1	93.5	132.1	128.2	106.9	285.0	269.7	224.1			
				14	15	17	43	43	43	44	43	40	7	6	2
6 girls	1 666	1 747	1 583	57.5	66.7	62.3	81.7	78.0	75.4	164.4	184.2	148.4			
				14	15	17	45	41	44	42	44	41	4	5	3
<i>Non diabetics</i>															
11 boys	2 858	2 810	2 484	86.7	93.5	84.5	129.6	123.9	107.8	318.5	310.0	298.6			
				12	14	14	41	41	39	46	46	49	11	10	11
4 girls	2 195	2 166	1 848	74.8	82.5	63.7	113.3	104.7	88.3	204.6	209.1	194.2			
				13	15	14	44	43	44	43	43	42	8	7	10

A careful inspection of the individual caloric intakes among diabetics and nondiabetics, divided according to age and sex, suggested that the present material as regards dietary composition would be representative for the whole group followed up.

The mean consumption of calories and the percentage distribution of protein, fat and carbohydrate are given in Table 1 for those 36 subjects who completed the three methods of assessing dietary composition. Refined carbohydrates (Table 1) were consumed on the average 0.8 times a day among diabetics and 1.8 times a day among nondiabetics.

The two 24 hour recalls showed very similar mean dietary consumptions within each group. The 7 day record gave lower caloric intake both among diabetics and non diabetics but an unchanged distribution on protein, fat and carbohydrate. The percentage of refined carbohydrates was considerably lower in the diabetics' own records than that obtained by the

interviews. No such difference was seen among nondiabetics.

The diabetic group had a somewhat lower caloric intake but a higher intake of protein in per cent of total calories than the comparable nondiabetic group. On the other hand the proportion of refined carbohydrates was lower among diabetics than among nondiabetics in respective of the method of assessment.

The results of the dental examination are given in Table 2. The diabetics had a lower frequency of caries but a higher frequency of supragingival calculus than the nondiabetics. Oral hygiene was similar in both groups.

The mean glycosuria was 28 g/25 hours (range 13-41) among diabetic boys and 35 g/24 hours (13-53) among diabetic girls. The mean fasting blood glucose was 280 mg/100 ml (100-430) among the boys and 330 mg/100 ml (230-490) among the girls. The individual data were plotted against the frequency of caries and calculus, but no sig-

Table 2 Dental status among diabetics and controls

Mean values \pm S.D. For units see text

Material	No of cases	Oral hygiene	Dental caries	Dental supragingival calculus
Diabetics	24	2.00 \pm 0.49	24.35 \pm 12.47	5.41 \pm 5.57
Controls	17	2.23 \pm 1.16	34.37 \pm 16.73	2.37 \pm 3.69
Significance		n.s.	$p < 0.05$	$p < 0.05$

FIBRINOGEN TURNOVER IN THE PREMATURE INFANT WITH AND WITHOUT IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

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The physiology and pathology of blood coagulation in the newborn and premature infant have gained in interest since a number of authors described intravascular fibrin clots in histological sections of various organs of infants dying in the neonatal period. These clots were considered to be the result of intravital intravascular coagulation (3, 5, 6).

The hypothesis originally set up by Stark et al (13) of a correlation between intravascular coagulation (i.v.C.) and idiopathic respiratory distress syndrome (IRDS) has been the subject of research in this hospital. In a series of programmes so far carried out no significant diminution of those factors usually considered to be consumed during i.v.C. (I, II, V, VII and platelets) could be demonstrated in uncomplicated IRDS (7, 10). In those infants who died of IRDS however these factors were diminished. A feasible explanation for this discrepancy between almost normal coagulation in infants with uncomplicated IRDS on the one hand and depression of coagulation potential with intravascular fibrin clots in infants dying of IRDS on the other would be that premature infants are able to compensate rapidly for loss of coagulation factors. Factor I (fibrinogen) appears particularly suitable for the investigation of this hypothesis as it is available as a pure substance tagged with radioactive ^{125}I for half-life determination studies.

MATERIALS AND METHODS

Patients

Ten premature infants aged between 1 and 9 hours were studied. Every infant received a small quantity of potassium iodide paste applied to the skin before the injection of ^{125}I fibrinogen and on 3 subsequent days in order to block the uptake of ^{125}I by the thyroid gland.

4 of the infants were healthy at the time they were chosen for the test and remained so (C 1380 g, B 1880 g, D 1900 g, Ö 2430 g). One of these (Ö) was not considered for calculation of fibrinogen half-life as it was discharged before the end of the test.

Six infants (K 1450 g, Ma 2340 g, Mu 1800 g, S 1670 g, NI and NII 1300 g) had the characteristic clinical and X-ray symptoms of IRDS and were treated in the usual manner with high ambient oxygen concentrations and infusion of glucose solutions containing sodium bicarbonate for correction of acidosis according to serial micro-Astrup measurements. Two of these infants (twins NI and NII) died before the fourth day of life. For comparison the half-life of ^{125}I tagged fibrinogen was calculated in an adult weighing 92 kg.

METHODS

^{125}I Fibrinogen. A commercial product was used (specific activity = 0.15 mCi/mg). In contrast to ^{125}I which has been most used hitherto ^{125}I is a soft γ ray emitter free of β radiation. The radiation dose for the tissues is therefore lower even taking into account the longer physical half-life. The premature infants received 5–15 μCi , the adult 85 μCi of ^{125}I fibrinogen applied intravenously.

Plasma fibrinogen determination. Blood sampling
Swiss Institute for Reactor Research CH Würenlingen

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FIBRINOGEN TURNOVER IN THE PREMATURE INFANT WITH AND WITHOUT IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

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METHODS

^{125}I Fibrinogen. A commercial product was used (specific activity $\sim 0.15 \text{ mCi/mg}$). In contrast to ^{125}I which has been most used hitherto, ^{125}I is a soft γ -ray emitter free of β radiation. The radiation dose for the tissues is therefore lower even taking into account the longer physical half-life. The premature infants received $5.15 \mu\text{Ci}$; the adult $85 \mu\text{Ci}$ of ^{125}I fibrinogen applied intravenously.

Plasma fibrinogen determination. Blood samples

Swiss Institute for Reactor Research, CH Würenlingen

Table 1 *Biological half life and calculated catabolic rate for ^{125}I fibrinogen in the adult and premature infant. Changes in total plasma fibrinogen concentration during the first days of life in premature infants*

Mean values and standard deviations were not calculated for the fibrinogen concentrations in this small series. For dynamics of plasma fibrinogen in the premature infant during the first day of life see Karitzky et al (10)

	Adult	Normal premature infants ($n=3$)			Premature infants with IRDS surviving ($n=4$)			
Half life (days)	4.5	2.8			2.1			
Catabolic rate (turnover/day) in percent	15.0	25.2			33.0			
		C	B	D	K	Ma	Mu	S
Total plasma fibrinogen (immunologically determined) in mg/100 ml before injection of ^{125}I fibrinogen								
After 24 hours	265	182	325	150	191	200	190	370
After 3 days	273	220	333	158	240	208	226	320
After 7 days	273	253	360	188	273	226	194	416
After 12 days	273	234	355	168	260	226	194	468
	273	235	334	200	290	234	208	460

was done at 15 and 30 min and at 1, 6, 12 and 24 hrs after injection later at daily intervals. 1.5 ml aliquots of blood were heparinised with an unweighed lyophilised crystal of ammonium heparinate (ROCHE). Fibrinogen determinations were carried out by radial immunodiffusion (9, 11).

^{125}I fibrinogen determination. 0.25 ml of plasma were incubated for 2 hrs at 37°C then for 24 hrs at 4°C with 0.2 ml monospecific anti human fibrinogen serum from the rabbit (8). The resulting immunoprecipitate was centrifuged at 15 000 g for 2 min and washed three times in buffered physiological saline. The radioactivity of all the precipitates was measured at the end of each experiment with a Packard Auto-Gamma Spectrometer (Model 3574). The activity 30 min after application of the ^{125}I fibrinogen was taken as 100%. The other measurements were expressed as ratios of this value after correction for zero emission.

External body measurements. For measurements over various organs a shielded Geiger Muller tube was used. In this way background interference was reduced four fold as compared with a scintillation counter. The detector tube was connected to a digital counter. The assay time was preset according to the expected impulse rate. The body measurements were taken in the following four positions:

1. Over the heart. Third intercostal space centred 3 cm from the sternal border.
2. In the right axilla. Pointing exactly towards the opposite axilla.
3. Over the right thigh. Over the diaphysis of the right femur and at 90° to this.
4. Over the right temporal region. On the midline between external orbita and external auditory meatus pointing exactly towards the opposite temporal region.

Statistical calculations. Arithmetical means were calculated for the ^{125}I fibrinogen activities measured between the third and twelfth day after injection. After demonstration of highly significant differences between the fibrinogen activity curve characteristics in the adult, the healthy prematures and the prematures with IRDS, variance analyses were carried out on the IBM 7040 computer in the Dept. of Maternal and University of Freiburg.

RESULTS

Fate of ^{125}I fibrinogen in adult

85 μCi of ^{125}I fibrinogen were injected intravenously and plasma disappearance rate was measured in the manner described above. It can be seen (Fig. 1 diagram 1) that after an initial rapid fall in concentration linear disappearance ensues at about 24 hours after injection (semi logarithmic scale). The half life is about 4.5 days or 108 hours corresponding to a catabolic rate of 15% per day. This result compares well with recent results in the literature (1, 4) whereas McFarlane (12) gives 74 hours as being the normal value. Assuming that the steep fall in activity during the first day is

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due to the distribution into the extravascular compartment, the intravascular fraction would be 40 after equilibration. This value is marked by the lower ordinate (the value is attained by extrapolation of the straight line during the second (steady) phase back to the ordinate)

¹²⁵I fibrinogen turnover in normal premature infants

The disappearance rate of ¹²⁵I fibrinogen in 3 normal premature infants can be seen in Fig. 1 diagram 2. All results are plotted and the curve points the mean values. It can be seen that a steady fall in activity is not attained before the 2nd to 3rd day after injection. From then on the function is a straight line when plotted on a semilogarithmic scale. The half life is 2.8 days from the third day of life on corresponding to a catabolic rate of 25.2 % per day. An assessment of fibrinogen metabolism during the first 3 days of life is not possible presumably

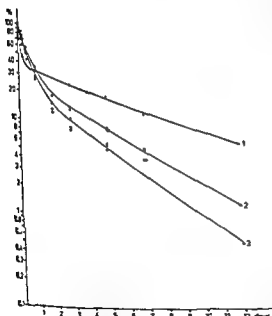


Fig. 1 Rate of elimination of ¹²⁵I fibrinogen from the plasma in an adult (curve 1) in 3 normal premature infants (curve 2) and in 4 premature infants with IRDS surviving (curve 3). The curves show the mean values, abscissa Days after injection of ¹²⁵I fibrinogen, ordinate ¹²⁵I fibrinogen activity in the plasma (as percent of the 30 min-value)

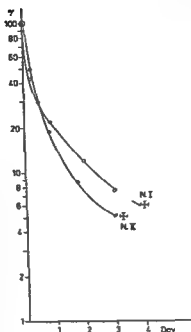


Fig. 2 Rate of elimination of ¹²⁵I fibrinogen from the plasma of premature twins dying of IRDS (NI and NII) abscissa Days after injection of ¹²⁵I fibrinogen and days of life, ordinate ¹²⁵I fibrinogen activity in the plasma (as percent of the 30-min value)

because a steady state is not reached. Analysis of the curves permits the tentative assumption of two consecutive functions, but the scatter is too large for certainty.

¹²⁵I fibrinogen turnover in infants with IRDS

Here again (Fig. 1 diagram 3) a steady state is not achieved before the third day of life. The half life of fibrinogen is 2.1 days corresponding to a catabolic rate of 33 % per day.

Variance analysis

For the period after equilibration (stable phase) following the third day orthogonal comparisons were carried out. There was a highly significant difference between the curves 1 (adult) and 2 (premature infants) (Fig. 1) the test quotient in the F test being 20.49 for $p < 0.001$. The difference between curves 1 and 3 (premature infants with IRDS surviving) was also highly significant ($p < 0.001$) the test quotient being 32.14. However there was no difference between curves 2 and 3 (test quotient 2.15).

Table 1 *Biological half life and calculated catabolic rate for ^{125}I fibrinogen in the adult and premature infant. Changes in total plasma fibrinogen concentration during the first days of life in premature infants*

Mean values and standard deviations were not calculated for the fibrinogen concentrations in this small series. F dynamics of plasma fibrinogen in the premature infant during the first day of life see Karitzky et al (10)

	Adult	Normal premature infants ($n=3$)			Premature infants with IRDS surviving ($n=4$)			
Half life (days)	4.5	2.8			2.1			
Catabolic rate (turnover/day) in percent	15.0	25.2			33.0			
		C	B	D	K	Ma	Mu	S
Total plasma fibrinogen (immunologically determined) in mg/100 ml before injection of ^{125}I fibrinogen								
After 24 hours	265	182	325	150	191	200	190	3 ¹
After 3 days	273	220	333	158	240	208	226	3 ¹
After 7 days	273	253	360	188	273	226	194	4 ¹
After 12 days	273	234	355	168	260	226	194	4 ¹
	273	235	334	200	290	234	208	4 ¹

was done at 15 and 30 min and at 1, 6, 12 and 24 hrs after injection. Later at daily intervals 15 ml aliquots of blood were heparinised with an unweighed lyophilised crystal of ammonium heparinate (ROCHE). Fibrinogen determinations were carried out by radial immunodiffusion (9, 11).

^{125}I fibrinogen determination. 0.25 ml of plasma were incubated for 2 hrs at 37°C then for 24 hrs at 4°C with 0.2 ml monospecific anti-human fibrinogen serum from the rabbit (8). The resulting immunoprecipitate was centrifuged at 15 000 g for 2 min and washed three times in buffered physiological saline. The radioactivity of all the precipitates was measured at the end of each experiment with a Packard Auto Gamma Spectrometer (Model 3575). The activity 30 min after application of the ^{125}I fibrinogen was taken as 100. The other measurements were expressed as ratios of this value after correction for zero emission.

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Statistical calculations. Arithmetical means were calculated for the ^{125}I fibrinogen activities measured between the third and twelfth day after injection. After demonstration of highly significant differences between the fibrinogen activity curve characteristics in the adult, the healthy premature and the premature with IRDS, variance analyses were carried out on the IBM 7040 computer in the Dept of Mathematics, University of Freiburg.²

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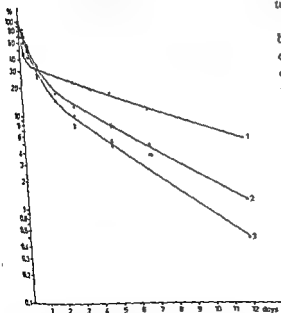


Fig. 1 Rate of elimination of ^{125}I fibrinogen from the plasma in an adult (curve 1) in 3 normal premature infants (curve 2) and in 4 premature infants with IRDS surviving (curve 3). The curves 2 and 3 join the mean values. Abscissa: Days after injection of ^{125}I fibrinogen. Ordinate: ^{125}I fibrinogen activity in the plasma (as percent of the 30 min value).

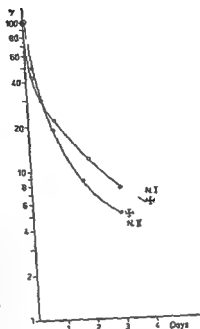


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brinogen molecule thus leading to rapid catabolism and false results in half life determinations. In order to exclude such an error the elimination of ^1I fibrinogen was determined in an adult as a control. The half life of 4.5 days calculated corresponds to the recent data in the literature. We conclude that the product used had been little altered by ^1I tagging.

Reference to the differing behaviour of fibrinogen in vivo in young and adult rabbits is made by Atencio et al. (2). These authors found a higher turnover rate in the young rabbits and explained this difference partly by the relatively large extravascular fraction of total fibrinogen partly by a higher catabolic rate.

Comparison of activity with the applied dose and body weight allows the assumption that the value 30 min after injection is representative for complete distribution and can be taken to be 100. Our results demonstrate a similarly increased extravascular fibrinogen fraction in premature infants as compared with the adult. Moreover either the influx into the extravascular compartment is slower or catabolism is increased during the first 2-3 days of life. An accelerated elimination of adult fibrinogen by the newborn infant must be taken into account considering the existence of a specific foetal fibrinogen (14). This is made improbable however by the fact that activity over head and thigh rises concomitantly with the fall in plasma activity (Fig. 3). These are areas with a relatively sparse vascularisation where the measured activity would presumably emit to a large degree from the extravascular compartment.

The interpretation of this result would be that ^1I fibrinogen slowly diffuses from the intravascular compartment and accumulates in the lymph until equilibrium is reached after 2-3 days whereupon curves 4 and 5 (head and thigh Fig. 3) run parallel to curve 1 (plasma).

In the body measurement shown in Fig. 4 (IRDS) as compared with Fig. 3 (normal premature) it is of particular interest that the activity in the head and thigh is turning to run

parallel to the other curves only after 24 hours. It is likely that this is due to the typical fibrinogen deposition in the hyaline membranes of IRDS.

Nothing can be said about half life and turnover during the first 2 days as the curves do not form a straight line (labile phase). Analysis of the curves seems to suggest a second exponential function but the scatter is too large for certainty. It must be assumed that fibrinogen turnover is rapid in this early phase as well.

From the third day on all three curves are straight (stable phase). Variance analysis proves highly significant differences in the catabolic rate in normal premature infants and those suffering from IRDS as compared with the adult (Fig. 1). According to the half life of 2.8 days a turnover of 25.2% per day can be calculated for normal prematures. Infants with IRDS recovering had 2.1 days and 33% turnover per day. In the adult only 15% of the fibrinogen pool is turned over. The catabolic rate in surviving prematures with IRDS is one third higher than that in normal prematures but the difference is not significant. Neither infant dying of IRDS showed peculiarities during the first 3 days of life (Fig. 2). On the third day after injection (third day of life) there was however very little remaining plasma ^1I fibrinogen activity (NI 7.6% / NII 5%). The first twin (NI) died of intraventricular haemorrhage. The massive clot had the same ^1I fibrinogen activity/mg fibrinogen as a plasma sample with the same fibrinogen concentration from the second day of life. It can be assumed that the main haemorrhage took place on this second day at a time when the clinical condition deteriorated with apnoea and symptoms of shock.

In both groups examined the fibrinogen level rose during the first three days of life and then remained constant in spite of the high catabolic rate of ^1I fibrinogen. This once again demonstrates the excellent regenerative capacity of even the premature newborn infant.

There is no insufficiency in liver synthesis of fibrinogen even in the infants with severe

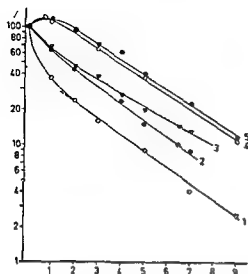


Fig 3 ^{125}I fibrinogen activity over various organs in a normal premature infant (D 1900 g) 1 Plasma 2 heart 3 right axilla 4 thigh 5 temporal area Abscissa Days after injection of ^{125}I fibrinogen and days of life

^{125}I -fibrinogen turnover in premature infants dying of IRDS

Male twins (N I and N II) were examined who died at the ages of 3.75 days and 3.0 days respectively. The first twin had massive intraventricular haemorrhage, the other had histologically verified intravascular fibrin clots in the vessels of the liver. The rate of ^{125}I fibrinogen disappearance was unusually high in these two infants. Premortal values were 7.6% (N I) and 5% (N II) of the original ^{125}I fibrinogen value (Fig 2).

Immunologically determined fibrinogen concentrations

The plasma fibrinogen values in the two groups of premature infants are shown in Table 1. It can be seen that a rise in concentration takes place in both groups during the first three days of life. This conforms with results achieved earlier showing a steady rise in fibrinogen concentration during the first day of life (10).

Measurement of ^{125}I -fibrinogen activity over various organs in normal premature infants

A typical example is given in Fig 3. The characteristic sharp fall in the ^{125}I fibrinogen plasma level in the first two days is apparent.

The half-life is 2.3 days. Activity over the heart and in the right axilla in which latter position mainly activity over the right lung is measured falls almost in a linear function when plotted semi logarithmically. In contrast to this, activity rises over the temporal region and over the thigh to values above initial up to the 24th hour. During the following days the curves continue almost parallel to that of ^{125}I fibrinogen in plasma.

Measurement of ^{125}I fibrinogen activity over various organs in infants suffering from IRDS

Fig 4 shows a typical curve for a premature infant with IRDS. Here again there is a rapid fall in ^{125}I fibrinogen activity during the first 2 to 3 days followed by a stable phase. The half life is 2.0 days. The values over the heart (2) and hand (5) correspond to those found in normal premature infants (Fig 3) however there is no rise in activity over the thigh.

Noticeably different from normal premature infants are the values over the right axilla (lung). A definite rise in activity takes place up to the 24th hour after injection.

DISCUSSION

McFarlane (12) was able to show that over iodimisation can produce changes in the fi-

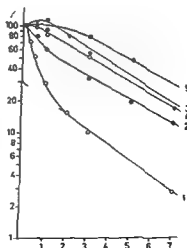


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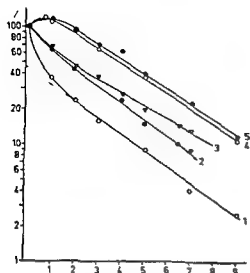


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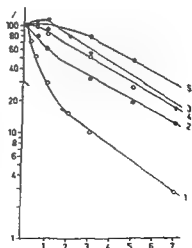


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parallel to the other curves only after 24 hours. It is likely that this is due to the typical fibrinogen deposition in the hyaline membranes of IRDS.

Nothing can be said about half life and turnover during the first 2 days as the curves do not form a straight line (labile phase). Analysis of the curves seems to suggest a second function but the scatter is too large for certainty. It must be assumed that fibrinogen turnover is rapid in this early phase as well.

From the third day on all three curves are straight (stable phase). Variance analysis proves highly significant differences in the catabolic rate in normal premature infants and those suffering from IRDS as compared with the adult (Fig. 1). According to the half life of 2.8 days a turnover of 25.2% per day can be calculated for normal prematures. Infants with IRDS recovering, had 2.1 days and 33% turnover per day. In the adult only 15% of the fibrinogen pool is turned over. The catabolic rate in surviving prematures with IRDS is one third higher than that in normal prematures but the difference is not significant. Neither infant dying of IRDS showed peculiarities during the first 3 days of life (Fig. 2). On the third day after injection (third day of life) there was however very little remaining plasma ^{125}I fibrinogen activity (NI 7.6% NI 5%). The first twin (NI) died of intraventricular haemorrhage. The massive clot had the same ^{125}I fibrinogen activity/mg fibrinogen as a plasma sample with the same fibrinogen concentration from the second day of life. It can be assumed that the main haemorrhage took place on this second day at a time when the clinical condition deteriorated with apnoea and symptoms of shock.

In both groups examined the fibrinogen level rose during the first three days of life and then remained constant in spite of the high catabolic rate of ^{125}I fibrinogen. This once again demonstrates the excellent regenerative capacity of even the premature newborn infant.

There is no insufficiency in liver synthesis of fibrinogen even in the infants with severe

IRDS Fibrinogen turnover in premature infants suffering from IRDS is, at least from the third day of life on, high enough to compensate for loss into the hyaline membranes and into intravascular clots. This means that lack of one of the classical signs of intravascular coagulation in the adult, namely hypofibrinogenemia, does not rule out DIC in IRDS. The turnover in these infants is more than double that in adults, thus allowing even a rise in plasma fibrinogen concentration during the course of the disease.

SUMMARY

Measurement of the elimination of small quantities of ^{125}I fibrinogen (5–15 μCi) demonstrates shortened half-life and higher catabolic rates in premature infants, and particularly in those infants suffering from IRDS compared with adults. Turnover in the adult is 15%, in the premature 25% and in the premature with IRDS 33% per day.

Equilibrium between intravascular and extravascular fibrinogen is not achieved before the third day of life. Later elimination is linear. There is a highly significant difference between ^{125}I -fibrinogen catabolism in the adult and the premature infant with and without the IRDS between the third and twelfth day.

Measurement of activity over various organs demonstrates that slow efflux of ^{125}I fibrinogen into the extravascular compartment causes a continuous fall in plasma activity during the first 2–3 days. In premature infants with IRDS the activity over the lungs rises during the first day of life. These results allow the conclusion that accelerated fibrinogen synthesis in infants with IRDS compensates for losses into the hyaline membranes and intravascular clots and even allows the physiological rise in plasma fibrinogen level during the first three days to take place.

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RUBIDOMYCIN IN BLASTIC PHASES OF ACUTE PROMYELOCYTIC AND MYELOBLASTIC LEUKEMIA IN CHILDREN

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The first reports on rubidomycin and daunomycin effectiveness in acute leukemia in 1966 (9, 13) were followed by many studies on its action in leukemias (1, 2, 5, 6, 7, 10) and tumours (8, 11, 12, 14). Only a few publications, however, deal with the use of rubidomycin in other than lymphoblastic forms of acute leukemia in children.

CLINICAL MATERIAL AND METHODS

Rubidomycin therapy was introduced in our clinic in 1967. In 25 months 21 courses of treatment have been administered to 11 children with other than lymphoblastic forms of acute leukemia. The diagnosis of acute leukemia was established on the basis of morphological and cytochemical criteria (peroxidase PAS).

The series includes 13 treatment courses in promyelocytic leukemia (4 cases), 5 courses in myeloblastic leukemia (4 cases) and single cases of erythroleukemia, acute monoblastic leukemia and acute myeloblastic transformation of chronic granulocytic leukemia. The age of the patients ranged between 1 and 14 years.

The blastic phases in which rubidomycin has been used are shown in Table 1.

The relationship between the disease period and rubidomycin therapy was as follows: shortly after the diagnosis of acute leukemia 5 courses of treatment were administered. Thereafter, in the first and second halves of a year of illness 8 and 8 courses respectively were administered and 2 years after diagnosis two courses.

Rubidomycin was usually given with prednisone in the dose 3 mg per kg body weight (13 courses) and less often alone. During remission treatment with 6-mercaptopurine was introduced, alternating with methotrexate. The treatment was supplemented by

low protein, purine free diet. (4) Rubidomycin was given in fast intravenous injections of 1 mg/kg body weight (mean dose 1.2 mg/kg) every second day (10 treatment courses) every 3 days (6 courses) or at longer intervals (5 courses).

RESULTS

The therapeutic results were assessed on the basis of the following criteria:

Complete remission

I Hematologic criteria: (a) peripheral blood disappearance of parablasts, $Hb \geq 65\%$, erythrocytes $\geq 3,500,000/\text{mm}^3$, granulocytes $\geq 1,500/\text{mm}^3$, blood platelets $> 150,000/\text{mm}^3$; (b) bone marrow parablasts and blast cells count $\leq 10\%$. II Clinical criteria: disappearance of all clinical symptoms of leukemia and leukemic infiltrates accessible to examination.

Partial remission

Separate decrease in the number of parablasts and blast cells in the bone marrow $\leq 10\%$.

Beginning in 1969 stricter criteria were adopted ($< 5\%$ parablasts and blast cells in myelograms). Earlier efforts had already been made to obtain more pronounced decrease of parablastosis in the bone marrow so that in 6 out of 11 remissions the bone marrow contained less than 5% parablasts and blast cells and in the remaining 5 cases percentages of these cells were less than 7.5%. The frequencies of remissions are shown in Table 2.

Table 1 *Rubidomycin courses in different blastic phases of the disease*

Number of blastic phases	Number of treatment courses
I-II	14
III-IV	5
V-VI	2
Total	21

In promyelocytic leukemias, remissions were observed even in late relapses of the disease, as shown in Table 3

The favorable results in relapses may be attributed partly to early diagnosis and introduction of the rubidomycin therapy before overrunning of the bone marrow by parablasts

Promyelocytic leukemia in children is not infrequently characterized by tumours of the head (3). It has been observed that the reappearance of exophthalmos or a tumour in the cranium is very characteristic of relapse in children with this form of leukemia and that it is invariably accompanied by blastic proliferation in the bone marrow. The bone marrow in relapses diagnosed in this way was not over-

run completely by parablasts. Cranial tumours were never observed in bone marrow remissions, which were always of short duration. The rapid rate of appearance of relapses was the reason for prolonged hospitalization.

In all cases the mean dose of rubidomycin per treatment course was low. Remissions appeared on the average 10 days after the beginning of treatment. In promyelocytic leukemia a greater number of relapses was treated so that the total dose of rubidomycin per child was larger (12.5 mg/kg body weight) than in other forms of acute leukemia (4.9 mg/kg).

Toxic symptoms. In 8 out of 21 treatment courses no side effects were observed. In the remaining treatment courses 26 harmful symptoms occurred including 19 pertaining to the blood. Neutropenia was observed in 10 treatment courses. In one half of courses a toxic neutropenia occurred alone, and less frequently it was encountered as a component of pancytopenia associated with thrombocytopenia, or combined only with anaemia. Marked neutropenia appearing in the course of treatment (2) was considered to be an indication to change the administration of rubidomycin.

Table 2 *Remissions in cases treated with rubidomycin*

Form of acute leukemia	Total number of treatment courses	Remissions total and partial	Percentage of total and partial remissions
Promyelocytic	13	8	61
Myeloblastic and others	8	3	37
Total	21	11	52.3

Table 3 *Number of remissions in successive blastic phases treated with rubidomycin*

Form of leukemia	Blastic phases					
	I-II		III-IV		V-VI	
	Treatment courses <i>n</i>	Remissions <i>n</i>	Treatment courses <i>n</i>	Remissions <i>n</i>	Treatment courses <i>n</i>	Remissions <i>n</i>
Promyelocytic	7	5	4	2	2	1
Myeloblastic and others	7	3	1	0	0	0
Total	14	8	5	2	2	1

Ulceration of the oral cavity was observed sporadically. ECG abnormalities were sometimes difficult to interpret. Three times the changes observed were attributed to the toxicity of rubidomycin. One child in a resistant phase died suddenly from cardiovascular failure which seemed to be connected with rubidomycin therapy.

DISCUSSION

Our observations indicate that rubidomycin is effective in those forms of acute leukemia in children in which remissions are otherwise very rare. This applies especially to promyelocytic leukemia which may be defined as "hyperacute" because of its exceptionally rapid course. Particular sensitivity of promyelocytic leukemia to rubidomycin therapy has been emphasized (1, 2, 15). The high percentage of remissions in promyelocytic leukemia including late relapses may be attributed to earlier diagnosis. The total dose of rubidomycin per treatment course was relatively low. A larger number of cures was carried out in promyelocytic leukemias during repeated blastic exacerbations so that the total dose of rubidomycin in this form per child (12.5 mg/kg body weight) was larger than used to be in the other forms of acute leukemia (4.9 mg/kg) in children.

In a majority of cases prednisone treatment was associated with the rubidomycin therapy. Combined therapy has been recommended by other authors (6, 7). In our experience simultaneous administration of rubidomycin, prednisone and low protein diet in acute lymphoblastic leukemia increases the percentage of remissions. In other morphological forms of the disease prednisone only modifies the intensity of harmful hematologic side effects of rubidomycin.

It should be emphasized that treatment with rubidomycin of acute leukemia, especially promyelocytic and myeloblastic, which are characterized by a tendency to neutropenia, requires utmost caution and ought not to be schematic.

Flexibility of the intervals between injections is safer than daily administration of rubidomycin. Before each injection the decision to continue the drug should be preceded by a detailed analysis of the patient's clinical state and blood picture. Frequent myelograms are desirable.

SUMMARY

Rubidomycin was administered in doses of 1 mg/kg body weight usually in combination with prednisone. The best results were obtained in promyelocytic leukemia. Promyelocytic leukemia in children seems to be characterized by sensitivity to rubidomycin even in late exacerbations.

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NITROBLUE TETRAZOLIUM REDUCTION BY NEUTROPHILS OF NEWBORN INFANTS IN IN VITRO PHAGOCYTOSIS TEST

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The process of phagocytosis induces metabolic changes in normal neutrophils. Before the intracellular killing of ingested organism takes place an increased proportion of glucose is metabolized via the hexose monophosphate shunt. There is also a marked increment in oxygen consumption and hydrogen peroxide production (7). Such metabolic events are accompanied by a reduction of nitroblue tetrazolium (NBT) dye (1).

Baehner & Nathan (1) and Windhorst et al (13) using NBT and a combined phagocytosis histochemical test showed that during *in vitro* phagocytosis of latex particles the capacity of normal human neutrophils to reduce the dye was significantly increased. Park et al (9) showed that a supravital method based on the *in vitro* reduction of NBT can be used to differentiate certain types of bacterial infection from non bacterial illnesses. During the course of a natural infection a large number of peripheral neutrophils will reduce NBT whereas a small proportion of neutrophils will reduce this dye without challenge. The spontaneous reduction of NBT by neutrophils was found to be increased in phagocytes of term (5-10) and premature (3-4) newborn infants in absence of bacterial infection thus resulting in a falsely positive NBT test.

The purpose of this study was to determine the NBT reducing ability of term and premature infant, and adult leukocytes employing

an *in vitro* phagocytosis system consisting of whole blood with *Pseudomonas aeruginosa* as test organism.

MATERIAL AND METHODS

The study groups consisted of 17 clinically normal pre term infants (premature) of gestational age less than 37 weeks (11-12) from 1 to 12 days old (weight range 880-2300 g), 6 pre term infants from 7 to 18 days old (weight range 980-2180 g) with known bacterial diseases (skin abscesses with bacteremia, septicemia, bacterial meningitis and pneumonia), 14 apparently healthy term infants from 12 hours to 23 days old (weight range 2540-4100 g) and 7 adults.

Venous blood was drawn under aseptic condition transferred into disposable capped plastic tube and 50 units of heparin (Boots) per ml of blood were added.

The histochemical NBT reduction test was carried out using the method of Park et al (9). Approximately 0.1 ml of blood was gently mixed with an equal amount of 0.2% NBT (Sigma) solution and 0.15 M phosphate buffered saline solution pH 7.2. The mixture was incubated at 37°C for 15 min and subsequently kept at room temperature for an additional 15 min. Coverslip smears were prepared, air dried and then counterstained with Wright stain (BDH). All slides coded and examined under the microscope with oil immersion were read by the same investigator (P.C.) who did not know the source of the cells. The percentage of neutrophils containing a large black deposit of formazan (reduced NBT) were classified NBT positive. NBT tests were performed in all the blood samples a few minutes after the drawing of blood and before adding bacteria.

The phagocytosis experiments were carried out in plastic tubes by adding bacteria to whole heparinized blood. *Pseudomonas aeruginosa* grown in Lowbury selective medium served as the test organism and was obtained from the stock culture collection at the

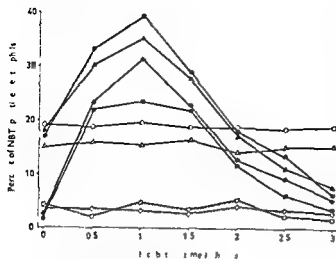


Fig 1 NBT reducing activity by leukocytes in phagocytosis experiments. Blood from premature (closed circles) and term (closed triangles) infants, premature infants with bacterial infections (closed squares) and adults (closed lozenges) incubated with *Pseudomonas aeruginosa*. Blood from premature (open circles) and term (open triangles) infants, premature infants with bacterial infections (open squares) and adults (open lozenges) without adding bacteria.

Pediatric Clinic (2). Bacterial suspensions were made from 24 hours old cultures washed twice with Hanks balanced salt solution and resuspended to a known concentration in saline solution. The inocula in the volume of 0.05 ml contained approximately 10^8 organisms. The number of test organisms in the original suspension was determined by plate count method on blood agar. The mixture consisting in 2 ml blood and bacteria was then incubated at 37°C in roller drum at 1/8 rpm. At 30 min intervals blood samples were removed and tested for the NBT reducing activity of leukocytes.

Control NBT reduction activity in blood samples without additional bacteria obtained from 9 healthy premature, 8 term infants, 6 premature infants with bacterial diseases and 7 adults was also determined.

All the blood samples including that of the controls from which bacteria were omitted were treated in the same way. A total of 74 blood samples obtained from 44 subjects were studied. The results of the NBT tests in the bloods of the premature, full term infants and adults were compared with those of controls.

RESULTS

Histochemical NBT tests were carried out on a series of premature infants, term infants and adults leukocytes at rest and during phagocytosis. The results of the NBT reducing activity by neutrophils from newborn infants and adults in *in vitro* phagocytosis tests are presented in

Fig 1 The percentage of NBT positive neutrophils in various groups are summarized in Table 1.

A significantly greater percentage of neutrophils incubated with *Ps. aeruginosa* reduces NBT if compared with the neutrophils of controls without additional bacteria ($p < 0.01$). The histochemical test determinations on blood samples taken at intervals after adding bacteria showed progressive increasing of NBT positive neutrophils. The mean percentage in premature and term infants was, at first reading, 17.6 ($SD \pm 6.78$) and 17.7 ($SD \pm 10.3$) respectively, then it rapidly rose reaching after 0.5 hour of incubation with *Ps. aeruginosa* 33.2 ($SD = \pm 14.9$) and 30.3 ($SD = \pm 17$) respectively. The greatest number of NBT positive leukocytes was found after 1 hour of incubation with a mean percentage of 39.4 ($SD = \pm 12.9$) in premature infants and 35.5 ($SD = \pm 16.5$) in term infants. In both groups the proportion of NBT positive neutrophils then progressively decreased to 6.5% ($SD = \pm 3.4$) and 8.1% ($SD = \pm 6.9$) after 3 hours of incubation. The percentage of NBT positive neutrophils in premature infants with bacterial diseases and adults was, at first reading 2.1 ($SD = \pm 1.4$) and 2.7 ($SD = \pm 1.7$) respectively. These mean values progressively increased reaching after 1 hour of incubation, 23.7% ($SD = \pm 13.9$) in premature infants and 31.5% ($SD = \pm 20.2$) in adults. In both groups the proportion of NBT positive cells then decreased to 6.2% ($SD = \pm 2$) and 3.5% ($SD = \pm 1.3$) respectively after 3 hours of incubation.

In the uninoculated blood controls the initial values of NBT positive neutrophils did not vary significantly with the incubation time. The mean percentage of NBT positive neutrophils ranged between 18.6 ($SD = \pm 11.2$) and 19.7 ($SD = \pm 10.6$) in premature infants, 3.2 ($SD = \pm 1.8$) and 5.1 ($SD = \pm 2.9$) in premature infants with bacterial infections, 14.1 ($SD = \pm 6.9$) and 16.6 ($SD = \pm 12.1$) in term infants and from 3 ($SD = \pm 1.1$) to 4 ($SD = \pm 2.3$) in adults.

Table 1 NBT reduction by neutrophils of newborn infants in *in vitro* phagocytosis test

			Percent NBT positive neutrophils at various time intervals of incubation (in hours)						
Age groups	No		0	0.5	1	1.5	2	2.5	3
Bloods incubated with <i>Ps aeruginosa</i>									
Premature infants	17	SD ±	17.6	33.2	39.4	28.8	17.8	13.5	6.5
			6.7	14.9	12.9	8.3	8.4	6.4	3.4
Premature infants with bacterial infections	6	SD ±	2.1	22	23.7	22	12.7	9.5	6.2
			1.4	11.6	13.9	9.9	3.5	5	2
Term infants	14		17.7	30.3	35.5	28.7	17.5	11.4	8.1
		SD ±	10.3	17	16.5	15.5	13	10.5	6.9
Adults	7		2.7	23.5	31.5	22.5	12	6.2	3.5
		SD ±	1.7	17.9	20.2	5.9	5.1	1.2	1.3
Uninoculated blood controls									
Premature infants	9		18.6	18.8	19.7	18.8	18.8	18.5	19.1
		SD ±	11.2	10.9	10.6	10.9	5.4	11.4	11.6
Premature infants with bacterial infections	6		3.2	2	4.7	3.1	5.1	2.2	2.1
		SD ±	1.8	1.5	1.9	2.1	2.9	1.8	1
Term infants	8		15.1	16.1	15.5	16.6	14.1	15	15.6
		SD ±	7.2	9.9	7.8	12.1	6.9	7.9	11.8
Adults	7		3	3.5	3.1	2.9	4	3.2	2.8
		SD ±	1.1	1.9	2.4	1.7	2.3	1.8	1.9

DISCUSSION

The results of the histochemical NBT test in *in vitro* phagocytosis experiments showed the expected rise of NBT reduction by phagocytes in neonatal and adult leukocytes. When incubated with *Ps aeruginosa* leukocytes from newborn infants and adults reduce NBT dye to a statistically significant greater extent than do resting control leukocytes without additional bacteria. These data confirm the results of Bachner & Nathan (1) and Windhorst et al (13) who reported that during phagocytosis the NBT reducing activity of normal human leukocytes was considerably increased.

In our studies the NBT test determinations on blood samples taken at 30 min intervals after adding bacteria showed a progressive increase of NBT positive neutrophils with the maximum rate of reduction after 1 hour of incubation at 37 C in all groups.

The mean percentage was at first reading premature infants 17.6 premature infants with bacterial infections 2.1 term infants 17.7 and adults 2.7. After 1 hour of incubation it was 39.4 23.7 35.5 and 31.5 respectively. These

mean values then decreased and reached their lowest degree after 3 hours of incubation.

On the contrary in the uninoculated control groups no definite increase of spontaneous NBT reduction activity was observed by resting neutrophils at the same incubation time even in the group of premature infants with documented bacterial infections. The mean percentage of NBT positive leukocytes did not vary significantly from the initial values.

We previously reported (3, 4) that leukocytes obtained from healthy premature and term infants reduce NBT to a greater extent than do neutrophils of premature infants with acute bacterial infections. The worsening of the infection is associated in these subjects with a decreasing of NBT reduction. On the other hand improvement and recovery were associated with an increase in the proportion of NBT positive neutrophils. The reason for the decreased NBT reduction activity in premature infants in natural infections but not in *in vitro* phagocytosis experiments remains to be explained. Preliminary study suggests that newborn leukocytes consume twice as much oxy

gen as control cells (10), but specific enzyme or enzymes involved in the reaction have certainly not yet been identified (6)

On the other hand in *in vitro* phagocytosis experiments, here reported, the values of the first reading agree with the already observed NBT reduction activity of the circulating leukocytes from neonates, tested without further *in vitro* challenge, particularly for the premature infants with bacterial infection. Although these findings may indicate that neutrophils are undergoing *in vivo* metabolic changes similar to those associated with *in vitro* phagocytosis, the incubation with *Ps aeruginosa* did not reveal a decrease of NBT reduction activity by leukocytes of premature infants.

It may be concluded that the reduction of NBT by neutrophils from neonates and adults significantly increases when they are incubated *in vitro* with *Ps aeruginosa* with the maximum rate of reduction after 1 hour of incubation. Furthermore a difference in behaviour between neutrophils of premature infants with bacterial infection during *in vitro* phagocytosis experiments and those phagocytosing *in vivo* during a natural acute infection of bacterial origin is suggested by the different rate of NBT reduction.

SUMMARY

Venous blood from healthy premature infants, premature infants with known bacterial infections, normal term infants and adults was incubated *in vitro* with *Pseudomonas aeruginosa*. At 30 min intervals, blood samples were tested for the nitroblue tetrazolium (NBT) reducing activity of neutrophils. The results of the histochemical NBT test were compared with those of the blood controls from which bacteria were omitted.

The stimulation of neutrophils with *Ps aeruginosa* induces a considerable increase of NBT reduction in all 4 groups of subjects examined. The maximum rate of NBT reduction was observed after 1 hour of incubation with the test organism, and reached its lowest degree after 3 hours. By contrast the NBT reduction by un-

stimulated neutrophils of the same subjects remain unchanged during a 3 hour period of incubation.

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DERMATOGLYPHICS AND THE SIMIAN CREASE IN INFANTS OF LOW BIRTH WEIGHT

A Pilot Study

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The dermal ridges are in their complete and permanent form by the end of the fourth month of intra uterine life (8) and the palmar creases are formed even earlier (6). Although dermatoglyphic features are primarily under genetic control the infant's developing dermatoglyphics may be altered by environmental factors acting on the fetus including maternal rubella (2) and thalidomide (8).

It is now accepted that many low birth weight (LBW) infants are born after a normal gestation period i.e. "Small for dates". These undergrown infants are probably associated with various causes including fetal malnutrition and hypoplastic growth disturbances (11).

This pilot study sought dermatoglyphic findings in a group of LBW infants on the premise that intra uterine disturbances leading to the premature birth of a "Small for dates" infant might also cause unusual dermatoglyphics. This study may be regarded as an extension of the work of Davies (5) who found an increased incidence of prematurity among neonates with a single transverse palmar crease.

MATERIAL AND METHODS

73 neonates aged 1-49 days, weighing less than 2000 g at birth, 33 males and 40 females, Jews and Gentiles.

¹ 1968 HEW Children's Bureau Senior Medical Student Fellow from the University of Florida School of Medicine Gainesville Florida.
Head of Paediatric Department.

Arabs formed the study (LBW) group. They included 3 pairs of twins and a further 11 single members of a twinship. All were apparently without malformations. They were examined in the premature units of the Rambam Hospital Haifa, the Central Emek Hospital Afula, the WIZO Babies Home Tel Aviv and the Kurya Maternity Hospital, Tel Aviv.

Both hands were examined by direct inspection with a magnifying lens under appropriate illumination. The following frequencies of features were analysed:

- Simian crease (simian line, palmar crease)
 - typical
 - transitional
- Interdigital patterns
- High axial triradius
- Hypothenar patterns
- Thenar patterns
- Finger patterns (loops, whorls, arches)

The criteria used for defining the dermatoglyphic features are according to those in the Memorandum on Dermatoglyphic Nomenclature (9). Axial triradius was defined as high when its position was $t \pm t$ or t . The schematic forms of typical and transitional forms of the simian crease as defined above are shown in Fig. 1 (12). "Typical simian crease" (Fig. 1/2) was recorded when there was a complete fusion of the usual two transverse palmar creases into one uninterrupted transverse line. "Transitional" forms of simian crease (Figs. 1/3 and 1/4) were recorded when the fusion of the 2 transverse lines was not complete or when they were clearly connected. Fig. 1/1 shows the normal form of palmar creases.

Two control groups were examined viz.

- 1 100 normal neonates by direct inspection of the hands for dermatoglyphic features and simian crease.
- 2 979 school children attending ordinary Haifa schools, by direct inspection of the palms for simian crease.



Fig 1 Forms of Simian line

RESULTS

The findings in the LBW infants and the control groups are summarised in Table 1

A higher frequency of simian crease was found among the LBW group. The difference in the total incidence of simian crease (typical and transitional) between the LBW group and the control neonate group was significant at the 5% level. For each type of simian crease, there was a significantly higher incidence of simian crease in the LBW group as compared with the large group of school children controls ($p < 0.05$ for each type and $p < 0.005$ for the total frequency of simian crease).

There was no significant difference in the frequency in dermatoglyphic features studied

Table 1 Incidence (%) of Simian crease and dermatoglyphic features in 73 low birth weight neonates and control groups

100 normal neonates and 979 school children

Pattern present	Control groups		
	73 LBW neonates	100 Normal neonates	979 School children
<i>Simian crease</i>			
Typical	5.4	1.0	2.0*
Transitional	10.9	5.0	4.7*
Total	16.3	6.0*	6.7**
<i>Dermatoglyphic features</i>			
High axial triradius	6.8	7.0	
<i>Palmar patterns</i>			
-hypothenar	16.5	15.0	
-thenar	1.3	1.0	
<i>Interdigital patterns</i>			
I	0.0	0.0	
II	1.3	4.0	
III	41.0	36.0	
IV	23.2	29.0	

Differences between LBW group and control group statistically significant at 5% level * and at 1% level **

Acta Paediat Scand 60

between the LBW group and the control neonate group

DISCUSSION

Many clinical disorders are known to be associated with an increased incidence of simian crease. Some authors have included transitional forms within their studies (2, 10) whereas others included only typical forms (1, 5).

Our previous study (4) described pedigrees showing the occurrence of the various forms of simian crease within the same family. This suggests that transitional forms are different expressions of the same entity. It seems justified therefore to group transitional and typical forms together in studies of simian crease frequency.

Since a significant number of LBW infants are small for gestation, the LBW group included Small-for-dates infants. The percentage of Small-for-dates infants within the LBW group has been estimated as 31% (3) or even as high as a half (7). Early intra uterine disturbances responsible for the birth of a Small-for-dates infant might also lead to unusual dermatoglyphic features in these infants.

Davies & Smallpiece (6) reported an increased incidence of prematurity among neonates with a typical simian crease. This association between simian crease and LBW neonates is confirmed by our findings.

No attempt was made to assess the duration of gestation in these 73 newborns. It would seem worthwhile to study the dermatoglyphic features of LBW infants with a known short gestation period, others with a known normal gestation period and relatives of these two groups of children.

SUMMARY

Dermatoglyphic features and simian crease incidence were studied in 73 neonates with a birth weight under 2,000 g. There was a higher frequency of simian crease, typical and transitional types, as compared with two control groups of 100 normal neonates and 929 school

children There was no significant difference in the incidence of dermatoglyphic features between the low birth weight and the control neonates

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CASE REPORT

HEPATIC INVOLVEMENT IN THE COURSE OF
ACQUIRED TOXOPLASMOSIS

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The characteristic clinical manifestations of congenital toxoplasmosis are found in the central nervous system, while acquired toxoplasmosis most frequently presents as generalized lymphadenopathy. Reports of visceral or abdominal toxoplasmosis are not numerous, indicating that this form is uncommon. According to Desmonts (2), splenomegaly is seldom found in children with acquired toxoplasmosis.

We present here the case history of a child suffering from hepatosplenomegaly and lymphadenopathy.

CLINICAL HISTORY AND FINDINGS

The patient J.S., a 5 year old girl, lived in the environs of Prague. Neither the family nor the personal history revealed anything relevant to toxoplasma infection. Eleven days before our patient fell ill, her three year old brother contracted measles with a typical clinical course. Apparently he became infected in a public nursery school (crèche) where measles epidemic had been attacking a number of children. The disease of our patient started on October 29 with prodromal catarrhal symptoms and appearance of typical maculopapular eruption on the skin. At the same time (November 1-2) she complained of abdominal pain, vomited repeatedly and so was referred to the Clinic of Infectious Diseases on November 2, 1966. On admission an acute abdomen was excluded. The typical appearances of measles were seen but nothing else. The liver, spleen and lymph nodes were all normal. During the next three weeks enlargement of liver and spleen developed together with a marked increase in serum glutamic oxalacetic acid transaminase (SGOT = 5.4 $\mu\text{mol/ml}$) and serum

glutamic pyruvic acid transaminase (SGPT = 10.0 $\mu\text{mol/ml}$). Eosinophilia was found in the peripheral blood. Throat culture showed the growth of β haemolytic streptococci and the antistreptolysin titre was elevated.

Since these changes do not occur in measles the patient was transferred to the Paediatric Clinic of the Institute for Post Graduate Medical Education in Prague on November 25, 1966. Rheumatic disease was suspected. The levels of serum transaminases were found to fall but the hepatosplenomegaly and the eosinophilia persisted. A low titre complement fixation test (CFT) did not suggest acute toxoplasmosis and the patient was discharged on December 7, 1966.

At follow up examinations the temperatures were normal but weakness and fatigue were pronounced. A second CFT showed a titre of 1/10 and so she was admitted to the Clinic of Infectious Diseases on January 13, 1967, where she remained until February 8, 1967. On admission the liver was palpable 4 cm below the right costal margin and the spleen was 3 cm below the left costal margin. The lymph nodes were enlarged in both posterior cervical chains in both axillae and in both inguinal regions. They varied in size from peas to cherries. They were tender, smooth of uniform consistency, well defined and mobile. They were covered by normal skin and were not painful on pressure.

While in hospital the temperature never rose above 37.6°C. Chest and skull X-ray showed no pathological changes. Ophthalmoscopic and electrocardiographic examinations revealed no abnormalities. There was no skin rash and no jaundice.

Laboratory investigations

Erythrocyte and leucocyte counts were within normal limits (3.2-4.2 million and 4700-10200/mm³ respectively). Haemoglobin 70 g/l, colour index 0.9. In differential counts the highest lymphocyte value was 57% and the highest eosinophile was 16% (Fig. 1).

Table 1 Liver function tests and serum transaminase activity*

Date	Total bilirubin (mg/100 ml)	Thymol turbidity units	SGOT ^a (μmol/l ml)	SGPT ^c (μmol/l ml)	Serum Fe (μg/100 ml)
Nov 4 1966	0.5	4.9	5.4	10.0	
Nov III 1966			1.4	1.7	
Nov 22, 1966			1.1	0.8	60
Dec 1 1966	0.4	2.4	1.1	0.4	
Jan. 16 1967		0.7	0.5	0.3	

* Determined by colorimetry based on the principles of Reitman & Frankel's method (*Am J Clin Path* 28: 56 1957)

^a Normal range of activity is 0.35 to 0.95 μmol/ml

^c Normal range of activity is 0.20 to 0.60 μmol/ml

No atypical lymphoid monocytes of the Downey type (glandular fever cells) were seen. Sedimentation rate (January 16 1967) was 12/18 mm (Westergren). Results of the liver function tests are recorded in Table 1.

Parasitological examination of faeces was repeated ten times and always yielded negative results. Examinations for brucellosis, listeriosis, leptospirosis and tularemia were negative. The Paul Bunnell test, serologic tests for syphilis and Dubos-Middlebrook test were negative and skin tests for tuberculosis were weakly positive. Urine contained neither protein nor bile pigments.

Serological data. Toxoplasma antibody tests were done 17 times over a period of 4 years. In three of these the titre was sufficiently high to indicate recently acquired and active toxoplasmosis. The first CFT titre was 10, the second 80 and the highest 3,200. The indirect fluorescent antibody test (IFAT) titres paralleled the CFT but at a higher level. The highest IFAT titre was 1:80. The highest titre of the Sabín-Feldman dye test was 4,000. During convalescence and afterwards the antibody titres fell as shown in Fig. 1. The technical details and interpretation of our serological procedures have already been recorded (7).

Skin test with our heat-treated toxoplasman (6) revealed a strongly positive reaction after 48 hours: erythema 35 × 30 mm 3+; induration 25 × 25 mm 3+.

An enlarged lymph node was removed from the left groin on January 17 1967. The capsule was markedly fibrotic and the peripheral lymph sinuses were packed with lymphoid cells. Proliferative epithelial cells of the reticulum were scattered diffusely throughout the periphery of the node. The germinal centres of the follicles were enlarged, active and contained many mitotic figures (Dr Válek, Dept of Pathology Inst for Postgrad Med Ed).

Mice inoculated with a suspension of the patient's lymph node developed toxoplasma antibodies in the first passage. However, toxoplasma could not be demonstrated in this or in subsequent passages.

Treatment

The child was given anti-toxoplasma treatment and then discharged in a good general condition afebrile

and symptomless. The liver and spleen decreased to 1.5 cm below the costal margin. The lymph nodes decreased to normal.

Subsequently the eosinophilia recurred and the raised CF titres persisted and so the child was given another course of treatment during a further stay at our clinic (June 15-30 1967). In both courses a triple combination of pyrimethamine (Daraprim, Burroughs Wellcome, England), sulfanilamido-methylpyrimidine (Sulfamethoxydin, Spofa, Czechoslovakia) and spiramycin (Rovamycin, Specia, France) was given. The total doses in these two courses were Daraprim 120 mg and 80 mg, Sulfamethoxydin 3,100 mg and 3,000 mg and Rovamycin 10 g and 7.5 g respectively.

During the two convalescence periods and since the patient has been repeatedly examined. The child appears to be well. The liver, spleen and lymph nodes are within normal limits. Liver function tests are normal and toxoplasma antibody titres are falling.

Epidemiological enquiries

Small domestic animals such as fowl and rabbits with which the child came into contact but not cats were kept in the patient's household. In the spring 1967 one of the rabbits died but the cause was not ascertained.

DISCUSSION

Splenomegaly, protracted fever and protozoa-like bodies (*Toxoplasma pyrogenes*) in the blood and spleen were reported by Castellani as early as in 1914. Karelitz (9, 10) distinguished two abdominal forms of postnatally acquired toxoplasmosis: firstly primary liver toxoplasmosis with transient initial enterocolitis without either involvement of peripheral lymph nodes or lymphocytosis and secondly primary cervico-nuchal or generalized lymphadenitis with lymphocytosis. In the latter liver involvement represents a late complication. In both

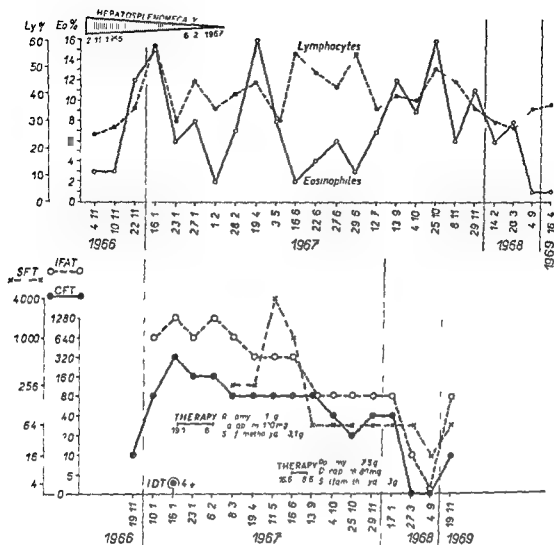


Fig. 1 Acquired toxoplasmosis with hepatosplenomegaly and eosinophilia in a 5-year-old girl. Toxoplasma antibody tests were done over a period of 4 years.

groups a tender enlarged liver, mild enlargement of the spleen, subicterus and an increased bilirubin level occur. Geyer (4) describes a series of adult patients with cervical lymphadenopathy and simultaneous liver involvement. In a subsequent report (5) Geyer describes the symptomatology of liver damage and the results of liver biopsies and also points out a possible relationship between toxoplasmosis and chronic liver damage. Liver involvement in patients with generalized toxoplasmosis has been recently reviewed by Theologides & Kennedy (23). Vischer et al (25) reported two cases of a disease resembling viral hepatitis associated with high titre of toxoplasma antibodies as well as the demonstration of parasites in sections of liver biopsy. Diamant-Berger (3)

Umdenstock & Raymond (24), Joseph et al (8) draw attention to abdominal symptoms in mesenteric lymphadenitis due to toxoplasma infection.

The pattern of serum transaminase activity in a newborn infant with jaundice due to congenital toxoplasmic hepatitis was described by Kove et al (11).

Silver & Dixon (21) demonstrated a marked eosinophilia of the peripheral blood and bone marrow in a 6-week-old infant with congenital toxoplasmosis. Lelong et al (13) reported eosinophilia in 6 cases out of 227 patients with acquired toxoplasmosis. According to Piquet et al (15) eosinophilia occurs more frequently in children than in adults. We were unable to find a cause for our patient's eosinophilia other

than toxoplasmosis. The recurrence of her eosinophilia during convalescence did not correspond with either the dynamics of her antibodies or with her clinical condition.

The histological features in glandular toxoplasmosis are not pathognomonic but are characteristic and well known from the reports of many authors (16, 17, 18, 19, 22). The most frequent changes described by them are filling of lymph sinuses with proliferative reticulum cells, proliferation of epithelioid cells either diffusely scattered or in clusters, follicular hyperplasia with large germinal centers, and basophilic granules (nuclear debris) situated either extra- or intra-cellularly. Demonstration of parasites is rare.

In our patient we found hepatosplenomegaly with evidence for liver damage, pluriglandular lymphadenopathy and eosinophilia. Rising antibody titres indicated the presence of toxoplasma infection. It could be questioned whether measles, toxoplasma infection or both were the cause of the actual symptoms from lymph nodes, liver, spleen and peripheral blood. In this connection the possible synergism of rubeola virus and toxoplasma parasite should be mentioned. It is well known among veterinarians that concurrent distemper is one of determining factors in the causal genesis of toxoplasma lesions in dogs. Koestner & Cole (quoted by Sum et al. 20) pointed out stress factors such as distemper which had been found to be responsible for activation of latent toxoplasmosis. Rubeola virus is antigenically similar to distemper virus (26). It can be believed that our patient acquired toxoplasmosis in a state weakened by concurrent or antecedent measles. There were no signs suggesting an activation of congenital toxoplasmosis. The source of infection may have been rabbits which in this country are almost all infected (12). A similar report of a child with a febrile illness, clinical evidence of pneumonia, generalized lymphadenopathy, hepatosplenomegaly with abnormal liver function tests and eosinophilia was made by Neumann et al. (14).

As may be seen either in the literature or

in our report the symptomatology of abdominal toxoplasmosis is not specific and can imitate other diseases. Subjective complaints (abdominal pain) and objective findings (hepatosplenomegaly, changes in liver tests) may be caused by many agents of disease. It is necessary to exclude appendicitis, viral hepatitis, infectious mononucleosis, other causes of mesenteric lymphadenitis and intestinal parasites in children. The results of diagnostic tests for toxoplasmosis are of paramount importance. It is not yet clear to what extent if any the activity of the infection can be related to eosinophilia. In our patient the eosinophilia persisted longer than the clinical signs and high toxoplasma antibody titres.

In conclusion we recommend that the possibility of toxoplasmosis should be considered in children with vague abdominal complaints and in those with hepatosplenomegaly.

SUMMARY

The authors report hepatosplenomegaly in a 5 year old girl together with pluriglandular lymphadenopathy, hepatic involvement and eosinophilia. Antibody tests for toxoplasmosis revealed high titres. The condition improved after treatment with toxoplasma-cidal drugs.

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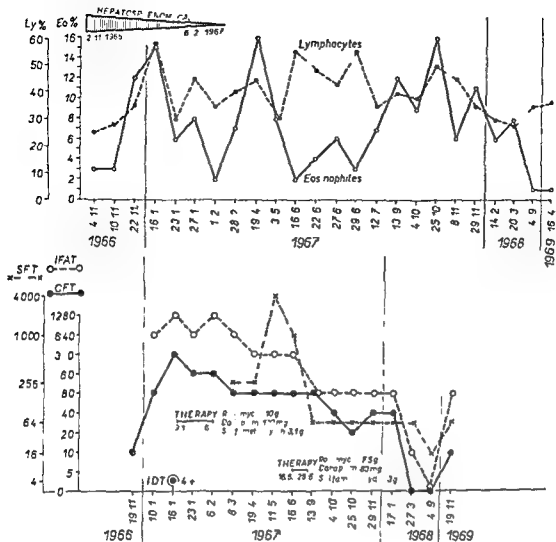


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In this issue of *Acta Paediat Scand* Kouba Jura and Zitova report on a case of toxoplasmosis and measles with appearance of hepatosplenomegalia and pronounced eosinophilia symptoms usually not seen in single infections with either one of the two agents. It should be noticed in this connection that measles virus is biologically closely related to distemper virus.

In searching for a mechanism responsible for the possible synergistic effect of measles virus and *Toxoplasma* the following circumstances should be considered.

Toxoplasmosis is accompanied by proliferation of phagocytizing reticulo-endothelial and large lymphoid cells both probably target cells in early stages of measles infection.

There is good evidence that in toxoplasmosis as well as in measles local antigen antibody reactions occur. In toxoplasmosis also circulating immune complexes appear. It is conceivable

that simultaneous formation of immune complexes locally in Arthus reactions and in the circulation may aggravate the inflammatory manifestations in spleen and liver sufficiently to produce symptoms.

Finally both infections are accompanied by lymphocytopenia which probably explains the reduction in immunological reactivity particularly cell mediated immunity.

The effect of double infection of lymphoreticular tissue is so far very little studied. The case presented by Kouba and coworkers indicates that this etiology sometimes is of clinical significance. It seems reasonable to assume that in the course of a long lasting infection like toxoplasmosis a patient may contract also a systemic virosis. In cases with unusual symptoms from the RES the possibility of a double infection should be kept in mind.

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Key words Hepatosplenomegaly lymphadenopathy toxoplasmosis

The Editors have asked dr Gunnar Hultdt to comment on the Case Report by Kouba et al

Like most other parasites *Toxoplasma* gives rise to long lasting infections. It can be demonstrated that the primary multiplication of the parasite takes place in reticuloendothelial and lymphatic tissue. Pronounced reactions from such tissue is one of the main characteristics of toxoplasmosis. Systematic studies on the duration of RES reactions in this infection are lacking, however.

A number of agents mainly of viral origin attack and cause reactions from RES. Double infections with *Toxoplasma* and one of these agents may act synergistically and give severe symptoms sometimes not recognizable in either one of the two infections involved.

Studies in progress indicate that such synergistic effect can be demonstrated in mice experimentally infected with *Toxoplasma* and Moloney virus. It is also well known among veterinarians that distemper in dogs runs a more severe course if the disease is complicated by infection also with *Toxoplasma*. Necroses in

th liver have been demonstrated in such cases but are usually not found neither in toxoplasmosis nor in distemper in dogs.

In this issue of *Acta Paediat Scand* Kouba Jira and Zitova report on a case of toxoplasmosis and measles with appearance of hepatosplenomegalia and pronounced eosinophilia symptoms usually not seen in single infections with either one of the two agents. It should be noticed in this connection that measles virus is biologically closely related to distemper virus.

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REVIEW ARTICLE

LACTOSE INTOLERANCE AND PROTEIN MALNUTRITION

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Milk is undoubtedly an extremely valuable source of essential nutrients, especially protein. The milk from most mammalian species, including man and cow, contains considerable amounts of a specific disaccharide, namely lactose. This disaccharide seems to stimulate the absorption of calcium in the small intestine, but to our knowledge no other physiological effect of lactose making it superior to e.g. glucose as a nutrient, has been demonstrated. In the small intestine lactose is hydrolyzed into glucose and galactose by small intestinal lactase. These two monosaccharides are absorbed into the blood stream by a common active transport mechanism and the galactose is rapidly converted into glucose by the liver.

During the last decade we have learnt that in some subjects the intestinal lactase activity is low or absent. The administration of lactose to adult subjects with lactase deficiency often leads to abdominal discomfort and diarrhoea. In infants with lactase deficiency more drastic symptoms will occur with watery diarrhoea which often leads to dehydration and severe malnutrition. Continued milk feeding may in such infants lead to death. Since the infants often have fever the symptoms may be mistaken as signs of intestinal infection.

Lactase deficiency may thus severely interfere with the use of milk as a nutrient both in infants and adults and since some forms of lactase deficiency are very common as we shall see below, this enzyme deficiency has to

be taken into account when milk is used as treatment for patients with different degrees of protein malnutrition. Protein malnutrition as such may also lead to lactase deficiency and in this way interfere with the intestinal hydrolysis of lactose. We will first describe the methods for diagnosis and the pathophysiology of lactose intolerance, and then the pathogenesis of the different forms of lactose intolerance. Finally we will discuss the therapeutic possibilities.

Methods of evaluating the intestinal disaccharidase activities

There are two important methods available namely oral tolerance tests and *in vitro* assay of the disaccharidase activities in biopsy specimens of the jejunal mucosa. In infants analysis of sugars in feces can also be of value for the diagnosis but in adults this analysis is of no value because unabsorbed sugar is consumed by the bacteria in the large intestine.

(1) *Oral tolerance tests* After oral administration of any of the common dietary disaccharides (dose 2 g/kg body weight or 30-50 g/m body surface) normal subjects will show a blood glucose peak with a maximum after 30-60 min an increase of more than 25 mg/100 ml above the fasting level. In a patient who is intolerant to the disaccharide in question the blood glucose curve will be flat (Fig 1) and the patient will get diarrhoea. A control tolerance test with the corresponding mono-

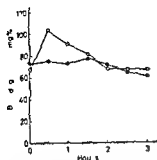


Fig. 1 Blood glucose curve during a lactose tolerance test (●) in a 2 1/2 year old boy with (congenital) lactose malabsorption. The curve is flat. Control tolerance test with a mixture of glucose and galactose (○) shows normal absorption of the monosaccharides (??)

saccharides is used to demonstrate that the monosaccharide transport is unimpaired (Fig. 1)

(b) Disaccharidase activity assay in biopsy

Table 1 Disaccharidase activities in homogenized biopsies of human small intestinal mucosa from one control group of North Americans and one group with lactose intolerance (20-24)

One unit of disaccharidase is the activity hydrolyzing 1 μ mole of substrate per min at 37 °C at pH 6.0. The activity has been calculated as units per g of protein

	Units per g protein			
	Control group (n=27)		Lactose intolerant group (n=12)	
	Mean	Range	Mean	Range
Maltase	266	111-470	234	94-505
Sucrase	87	26-138	77	19-194
Isomaltase	97	21-183	83	25-202
Lactase	44	9-98	2	0-6

specimens. For this examination a piece of small intestinal mucosa (usually taken at the duodeno-jejunal flexure) is obtained by peroral

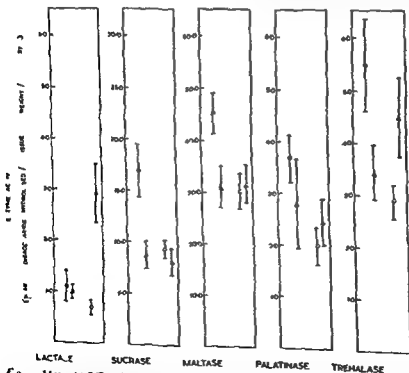


Fig. 2 Mean (\pm SD of the mean) of disaccharidase levels in jejunal biopsy specimens. Results for Baganda (●) and Bahutu (Rwanda) (○) children who had previously had kwashiorkor and for Baganda (▲) and Bahutu (Rwanda) (Δ) controls. The Bahutu are a mixed group of Bantu and Hamitic (Batutsi)

background and they normally retain high activity of intestinal lactase throughout life. The Baganda tribe has a very high incidence of (racial) lactase deficiency after the age of 4 years. From Cook & Lee (16)

REVIEW ARTICLE

LACTOSE INTOLERANCE AND PROTEIN MALNUTRITION

A DAHLQVIST and B LINDQUIST

*From the Research Department I E Block and the Paediatric Department
University Hospital Lund Sweden*

Milk is undoubtedly an extremely valuable source of essential nutrients, especially protein. The milk from most mammalian species, including man and cow, contains considerable amounts of a specific disaccharide, namely lactose. This disaccharide seems to stimulate the absorption of calcium in the small intestine, but to our knowledge no other physiological effect of lactose making it superior to e.g. glucose as a nutrient has been demonstrated. In the small intestine lactose is hydrolyzed into glucose and galactose by small intestinal lactase. These two monosaccharides are absorbed into the blood stream by a common active transport mechanism, and the galactose is rapidly converted into glucose by the liver.

During the last decade we have learnt that, in some subjects, the intestinal lactase activity is low or absent. The administration of lactose to adult subjects with lactase deficiency often leads to abdominal discomfort and diarrhoea. In infants with lactase deficiency more drastic symptoms will occur with watery diarrhoea which often leads to dehydration and severe malnutrition. Continued milk feeding, may in such infants lead to death. Since the infants often have fever, the symptoms may be mistaken as signs of intestinal infection.

Lactase deficiency may thus severely interfere with the use of milk as a nutrient both in infants and adults, and since some forms of lactase deficiency are very common, as we shall see below, this enzyme deficiency has to

be taken into account when milk is used as treatment for patients with different degrees of protein malnutrition. Protein malnutrition in such may also lead to lactase deficiency and in this way interfere with the intestinal hydrolysis of lactose. We will first describe the methods for diagnosis and the pathophysiology of lactose intolerance, and then the pathogenesis of the different forms of lactose intolerance. Finally we will discuss the therapeutic possibilities.

Methods of evaluating the intestinal disaccharidase activities

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(1) *Oral tolerance tests* After oral administration of any of the common dietary disaccharides (dose 2 g/kg body weight, or 30-50 g/m body surface) normal subjects will show a blood glucose peak with a maximum after 30-60 min an increase of more than 25 mg/100 ml above the fasting level. In a patient who is intolerant to the disaccharide in question the blood glucose curve will be flat (Fig 1) and the patient will get diarrhoea. A control tolerance test with the corresponding mono-

Table 3 Disaccharidase activities in intestinal mucosa from 33 kwashiorkor patients (35) compared with normal values obtained from children (10) and adults (24)

	Units/g protein (mean and range)		
	Normal values		
	Kwashiorkor patients	I children	II adults
Lactase	7 (1-17)	88 (14-132)	44 (9-98)
Sucrase	35 (5-71)	95 (32-228)	87 (26-138)
Isomaltase	51 (3-118)	89 (31-177)	97 (21-183)
Maltase	100 (0-226)	260 (83-615)	266 (111-4 0)

the carbohydrate composition of the food. Dietary lactose has no influence whatsoever on the intestinal lactase activity. Sucrose (and fructose) exert some influence on the sucrase and maltase activities but this influence is rather small and does not seem to be the major regulating factor (36). As we shall see below, some humans retain the high lactase activity through life but there are also large groups of humans which lose their lactase after the first years of life. In man as in animals dietary lactose does not influence the lactase activity of the mucosa (36).

Different forms of lactase deficiency

There seems to exist three different forms of lactase deficiency, all probably differing in their pathogenesis.

(1) Congenital hereditary lactase deficiency

The intestinal lactase is missing from birth and the enzyme defect lasts throughout life. This is supposed to be an inborn error of metabolism although it has not been definitely proven. At any rate it is extremely rare as is also the "severe lactose intolerance with lactosuria". These forms of lactose intolerance will therefore not have to be taken into account when the suitability of milk as a nutrient for large scale programmes is discussed.

(b) *Racial lactase deficiency* The first groups of humans used for studies on intestinal disaccharidases happened to be North Americans and North Europeans. In these groups high intestinal lactase activity was found in general to persist throughout life. When the studies were extended to other populations however it was discovered that in many races nearly all of the adults were lactose intolerant. This has e.g. been found true of Indians, Greenland Eskimos, Orientals etc. (Table 2). In Africa it was revealed that Negroes of Bantu tribes were lactase deficient as adults while subjects of Hamitic origin retained their lactase activity throughout life. This form of lactose intolerance thus seems to be bound to certain races or ethnic groups and it is usually named racial lactose intolerance.

Adult subjects of those populations with racial lactase deficiency will not be fit to consume milk. As infants however they possess intestinal lactase. Up to a certain age it will thus be possible to feed these subjects milk. It is then important to know when their intestinal lactase disappears. Cook (13) has performed a careful investigation in Baganda infants, this being one of the tribes showing racial lactase deficiency. Lactose tolerance tests were performed at different ages. The blood glucose response to lactose decreased gradually during the first years of life and in some exceptional cases lactose intolerance had already developed at six months of age. The racial loss of intestinal lactase thus occurs rather early in childhood. At the most can it be assumed that these subjects tolerate lactose during the first year of life. The variation with age has however so far only been studied in this single population.

It should be noted that this early loss of the tolerance for lactose occurred also in subjects which were well nourished and free from intestinal diseases. As will be presented below, malnutrition and diseases involving the small intestine can also influence the lactase activity.

(c) *Secondary lactase deficiency* Inflammatory and degenerative diseases involving the

Table 2 Frequency of adult lactase deficiency in some different population groups

Group studied	n	with lactose intolerance	Authors
<i>White</i>			
USA	(16)	6	Dunphy Littman Hammond Forstner Dahlqvist & Crane (24)
	(50)	16	Sheehy & Anderson (37)
	(19)	16	Cuatrecasas Lockwood & Caldwell (17)
	(20)	5	Bayless & Rosenzweig (4)
	(100)	6	Newcomer & McGill (34)
Switzerland	(18)	17	Auricchio Rubino Landolt, Semenza & Prader (3)
England	(69)	20	McMichael Webb & Dawson (33)
Denmark	(700)	5	Gudmand-Hoyer Dahlqvist & Jarnum (27)
Sweden	(400)	< 1	Berg Dahlqvist Lindberg Meeuwisse & Nordén (5)
Finland	(248)	15	Jussila Isokoski & Launiala (31)
<i>Negro</i>			
USA	(41)	73	Cuatrecasas Lockwood & Caldwell (17)
	(20)	70	Bayless & Rosenzweig (4)
Africa			
Bantu	(52)	91	Cook & Kajubi (15)
Other tribes	(60)	9-44	Cook & Kajubi (15)
Greek Cypriots	(17)	88	McMichael Webb & Dawson (33)
<i>Indians</i>			
USA	(3)	67	Welsh Rohrer Knudsen & Faustian (39)
<i>Oriental</i>			
Australia	(20)	95	Davis & Bolin (23)
USA	(11)	100	Chung & McGill (11)
<i>Greenland Eskimos</i>			
Denmark	(32)	72	Gudmand-Hoyer & Jarnum (28)

biopsy The mucosa is homogenized and analysed for disaccharidase activities (18). The activities are calculated as units (μ moles of substrate hydrolyzed per min at 37°C) either per g of protein (Table 1) or per g wet weight of the mucosa (Fig. 2). The correlation between the disaccharidase activity found in a mucosal biopsy and the results of oral tolerance tests is very high.

The normal range of mucosal disaccharidase activity

The range of disaccharidase activities in mucosal biopsy specimens from one control group of adult North Americans and one group with lactase deficiency is seen in Table 1.

In most patients with lactase deficiency a low residual lactase of a few units per g protein is found (Table 1). Part of this residual activity is caused by a lysosomal β galactosidase

which is probably not involved in the digestion of lactose (1, 2). In many patients, however, there is also a small residue of the brush border lactase (1). It is therefore important to define the limit of lactase activity below which the patient is intolerant to lactose. In the investigation shown in Table 1 none of the lactose-intolerant subjects had higher lactase activity than 6 units per g protein while none of the control subjects had less than 9 units. Other investigations have produced similar figures.

The newborn infant seems to have the same proportions of the different disaccharidase activities as the adult controls shown in Table 1. In this respect man seems to differ from most animals, in which the lactase activity is high at birth, while the other disaccharidases are low or absent. After weaning the animals lose their lactase and the other disaccharidases develop. This development is *not* regulated by

spect Kerpel-Fronius Jami & Fekete (32) have studied 62 Hungarian children with different degrees of malnutrition. Only 4 of these were classed as kwashiorkor patients. Oral tolerance tests showed that 75% of these malnourished children did not absorb lactose normally. The frequency and the severity of the lactose malabsorption seemed to increase with the severity of the malnutrition. Similar experience is reported by Berkel, Kiran & Say (6). These investigations seem to indicate that a milder degree of malnutrition may also interfere with the intestinal digestion of lactose. Cook (14) stated that in mild kwashiorkor mucosal damage and fall in disaccharidase activity are less common than in severe kwashiorkor but nevertheless patients with mild kwashiorkor often develop permanent lactase deficiency too.

Use of milk for treatment of protein malnutrition

The lactose intolerance in severe protein-calorie malnutrition plus the frequent occurrence of racial lactase deficiency in those parts of the world where protein malnutrition is commonly found seems to make the value of milk administration in its present form somewhat doubtful. Although patients with protein deficiency will benefit from milk administration they will also experience intestinal symptoms provoked by its lactose content.

How then can these difficulties be overcome? One apparent possibility is to replace milk by some other (vegetable) protein source of similar quality. The carbohydrate source should then be monosaccharides, sucrose, maltose or starch. The intestinal sucrase and maltase activities are more powerful than the lactase and there is no racial deficiency in these enzymes. It is therefore to be assumed that the patients with lactose intolerance due to various causes will be able to hydrolyze and absorb sucrose and maltose. Nevertheless this should be tested in the patients before any large scale production of such nutrients is commenced.

Another possibility is to remove the lactose from milk. Some attempts have been made to

remove lactose by dialysis but we do not regard this as a suitable method as it entails the loss of almost half of the calories. Furthermore there is a risk that other important components of the milk may be removed as well. Hydrolysis of the lactose into monosaccharides before the milk is fed should be a better proposition. It is possible to add fungal lactase preparations to milk and preincubate the mixture before it is fed (12, Dahlqvist & Meeuwisse—unpublished). This is however a too impractical and expensive method to be used on a large scale. We have therefore tried to hydrolyze the lactose in milk by a continuous process utilizing insoluble lactase built into a perfused column (19). This appears to be a possible way although all practical details are not yet solved. With this system the lactose in milk could be hydrolyzed during processing at the dairy in an equally simple way as Pasteurization is now performed. This method would have the advantage that no nutrients are removed from the milk and no additions (of fungal extracts) are made since the enzyme is not released from the column.

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small intestinal mucosa often cause a secondary decrease of all the disaccharidase activities. This is seen e.g. in tropical and non tropical sprue (21, 26, 40). It has often been observed that, although this secondary decrease affects all the disaccharidase activities the lactase activity is usually proportionally more depressed than the others. Since in normal lactose tolerant subjects the lactase activity is already weaker than the other disaccharidase activities (Table 1) it is natural that patients with secondary disaccharidase deficiency become primarily intolerant to lactose, and only in very pronounced cases do digestive disorders of other disaccharidases occur.

After treatment of the primary disease, the disaccharidase activities usually return to normal values. It seems however, as if in some cases the secondary lactase deficiency may become permanent. Gray, Wiltner & Colver (26) found that 63% of the patients with apparently cured tropical sprue showed lactose intolerance, as opposed to 21% in a control material.

Moreover in severe protein calorie malnutrition (kwashiorkor) there is a secondary disaccharidase deficiency affecting the lactase activity more severely than the other disaccharidase activities (7, 16, 25). The lactase deficiency in these patients has been demonstrated both by oral tolerance tests and by the assay of enzyme activities in mucosal biopsy preparations. The change from milk to carbohydrate-free diet has been reported to produce a dramatic drop in stool weight in these patients (8, 9). Monosaccharides and other disaccharides (sucrose and maltose) were well tolerated and it was therefore concluded that the lactase deficiency is a significant factor in causing the diarrhoea of kwashiorkor. The lactose intolerance in kwashiorkor has been demonstrated by many other authors as well (29, 30, 35, 38) (Table 3). Although reduced activity of the other disaccharidases and of monosaccharide transport can be demonstrated in patients with kwashiorkor (29, 30), the decrease in these activities will not result in sugar intolerance symptoms in most cases. This is due to two

factors: 1) in the normal intestine there is much higher activity of the other disaccharidases (and presumably also of the glucose transport system) than of the lactase, and 2) the lactase activity for some unknown reason seems to be proportionally more depressed than the other activities in conditions causing secondary disaccharidase deficiency.

It has to be pointed out that, in spite of the lactose intolerance of the kwashiorkor patients, they will benefit from milk administration (35). This has been observed by many different authors studying these problems. However, the milk administration will give diarrhoea and abdominal discomfort, and furthermore it is very probable that the rapid passage of the milk through the intestine will result in sub-optimal utilization of the protein as well as the other nutritional components of the milk.

It should also be observed that the pathological changes in the small intestinal mucosa of kwashiorkor patients will persist for a very long time after the introduction of dietary treatment and the lactose deficiency may often become permanent. Stanfield, Hutt & Tunnicliffe (38) found marked morphological alterations and low disaccharidase activities for up to 1 year after the onset of therapy, although clinical improvement had occurred within a few weeks. Prinsloo, Wittmann, Pretorius, Kruger & Feltham (35) have reported similar observations. Cook & Lee (16) studied patients cured of kwashiorkor 4-10 years before the time of the investigation. In tribes which normally have high lactase activity throughout life the subjects cured of kwashiorkor had low lactase although the activities of the other disaccharidases were high (Fig. 2). Apparently the subjects previously suffering from kwashiorkor had in this case a permanent lactase deficiency.

There is thus no doubt that patients with severe kwashiorkor will be intolerant to dietary lactose and that this intolerance will frequently become permanent also in populations in which normally a high level of lactase is maintained throughout life. We are less knowledgeable about milder forms of malnutrition in this re-

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Sixth Meeting June 11-13 1970 Turku Finland

MAIN TOPIC I

ANORECTAL SURGERY

G Grotte (Uppsala Sweden) *Introductory survey*

A brief classification of ano-rectal anomalies was followed by an outline of the procedures employed by Stephens Rehbein and Kiese wetter. Some of the author's own modifications of these methods were described. A cine film was shown of a case of high anal atresia in which the dorsal subsacral approach was used.

O Knutrud (Oslo Norway) *Anal atresia a 10 year series*

From 1959 to 1968 inclusive altogether 105 patients were treated for anal atresia at the Children's Surgical Department of Rikshospitalet Oslo. Three of them had already been operated on elsewhere so that the patients to whom we gave primary treatment totalled 102. Of these 27 had high and 75 low atresia.

In the high atresia group 7 patients died (25%) and in the low atresia group there were also seven deaths (9%). The combined mortality was thus 15%.

Other malformations were found in the high atresia group in 75% of cases and in 40% of the low atresia group. Seven of the 9 patients who died at operation had serious multiple defects.

Among the patients with low atresia 17 cases of vestibular atresia were found and 39

cases of perineal fistula—21 of these being boys and 18 girls.

A two-stage perineal plastic operation for cases with low atresia was described.

Inga Freiberg (Tallinn Estonian SSR) *Diagnosis and treatment of ano rectal malformations*

The frequency of ano-rectal malformations in the author's series was rather high, one case in 3 200 births. In this condition surgical repair has to be individual for every patient in accordance with the various anatomical and clinical findings. The achievement of sphincter control is of major importance.

In the years 1965-1969 10 children with ano-rectal malformations were treated in the Department of Paediatric Surgery at the Republic Hospital Tallinn. The report contained data on the various forms of the malformation, the sex distribution, the clinical picture and the diagnostic methods. The methods of surgical treatment were discussed in relation to the anatomical and clinical findings. The results were satisfactory.

B Thomasson & S Dahlstrom (Turku Finland) *Imperforate anus. Comments on the cases seen at Turku University Hospital 1954-1969*

Since the introduction of paediatric surgery as an independent subspecialty in this hospital 60 patients with imperforate anus have received primary surgical treatment here.

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- 28 Gudmund Høyer E & Jarnum S Lactose malabsorption in Greenland Eskimos *Acta Med Scand* 186 235 1969
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- 36 Rosenzweig N S & Herman R H Diet and disaccharidases *Amer J Clin Nutr* 27 99 1969
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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Sixth Meeting June 11-13, 1970 Turku Finland

MAIN TOPIC I

ANORECTAL SURGERY

G Grotte (Uppsala Sweden) *Introductory survey*

A brief classification of ano-rectal anomalies was followed by an outline of the procedures employed by Stephens Rehbein and Laesewetter. Some of the author's own modifications of these methods were described. A cine film was shown of a case of high anal atresia in which the dorsal subsacral approach was used.

O Knutrud (Oslo Norway) *Anal atresia a 10 year series*

From 1959 to 1968 inclusive altogether 105 patients were treated for anal atresia at the Children's Surgical Department of Rikshospitalet, Oslo. Three of them had already been operated on elsewhere so that the patients to whom we gave primary treatment totalled 102. Of these 27 had high and 75 low atresia.

In the high atresia group 7 patients died (25%) and in the low atresia group there were also seven deaths (9%). The combined mortality was thus 15%.

Other malformations were found in the high atresia group in 75% of cases and in 40% of the low atresia group. Seven of the 9 patients who died at operation had serious multiple defects.

Among the patients with low atresia 17 cases of vestibular atresia were found and 39

cases of perineal fistula—21 of these being boys and 18 girls.

A two-stage perineal plastic operation for cases with low atresia was described.

Inga Freiberg (Tallinn Estonian SSR) *Diagnosis and treatment of ano-rectal malformations*

The frequency of ano-rectal malformations in the author's series was rather high—one case in 3 200 births. In this condition surgical repair has to be individual for every patient in accordance with the various anatomical and clinical findings. The achievement of sphincter control is of major importance.

In the years 1965-1969 10 children with ano-rectal malformations were treated in the Department of Paediatric Surgery at the Republic Hospital Tallinn. The report contained data on the various forms of the malformation, the sex distribution, the clinical picture and the diagnostic methods. The methods of surgical treatment were discussed in relation to the anatomical and clinical findings. The results were satisfactory.

B Thomasson & S Dahlstrom (Turku Finland) *Imperforate anus. Comments on the cases seen at Turku University Hospital 1954-1969*

Since the introduction of paediatric surgery as an independent subspecialty at this hospital 60 patients with imperforate anus have received primary surgical treatment here.

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- 7 Bowie M D Barbezat G O & Hansen J D L Carbohydrate absorption in malnourished children *Amer J Clin Nutr* 20 89 1967
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- 13 Cook G C Lactase activity in newborn and infant Baghdad *Brit Med J* 1 527 1967
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necessary in the 277 cases subjected to operation since myectomy has only been performed for two-and-a-half years

Even so the number amounting to 10% at most shows that in all operations in which sphincter myectomy is performed as a matter of routine this is done unnecessarily in 90% of the cases in which a simple sphincter dilatation would have been adequate

U Schafer (Bremen BRD) *The abdomino-sacro-perineal approach in the treatment of the high supralevator type of imperforate anus*

At the Surgical Clinic of the Children's Hospital Bremen 96 children with a supralevator type of imperforate anus have been treated 76 cases were low supralevator forms and 20 high supralevator

In 76 of these 96 cases the abdomino-perineal approach was used and in 20 cases the abdomino-sacro-perineal approach. As the results by the abdomino-perineal approach were not very satisfactory in the high supralevator type we changed over to the abdomino-sacro-perineal approach in these cases. We regard this as the best way to find the exact position of the pubo-rectal sling and consider that children with a high supralevator type of imperforate anus will benefit from the additional approach. Our intention is to restrict the combined procedure to high levatory types only and to treat the low or translevator types by abdomino-perineal pull through without using the sacral approach

MAIN TOPIC II

CARDIOVASCULAR SURGERY ON INFANTS

J Crafoord G S ttergren & S Soderlund (Stockholm Sweden) *Determination of oxygen saturation in the pulmonary artery during banding*

Constriction of the pulmonary artery known as banding is performed in patients with VSD

with or without associated vitium cordis in whom cardiac insufficiency does not alter after treatment with digitalis and diuretics. The aim is partly to reduce the left to right shunt so that the insufficiency is removed and partly if possible to prevent any increase in pulmonary vascular resistance

How tightly should the band be drawn? Various methods can be employed to arrive at an answer to this question. It can be tightened until the heart stops and then released. Alternatively the pressure in the pulmonary artery can be reduced to a certain level. Or an attempt can be made to measure the flow with an electronic flow meter applied distally of the band

We have chosen a fourth method. Our working hypothesis is that an endeavour should be made to achieve normokinetic pulmonary blood flow with basal metabolism. In pre-operative catheterisation high oxygen saturation in the pulmonary artery indicates hyperkinetic pulmonary circulation. Thus it seems best to tighten the band at operation until a normal value for oxygen saturation is reached. We have done this in a number of cases. However the apparatus employed for measuring the oxygen saturation could have been more accurate. The patients were anaesthetised with halothane-oxygen-nitrous oxide. Blood samples were taken with a fine needle the mean pressure being registered at the same time. After the operation the patients reacted favourably and the cardiac insufficiency was found to have disappeared. Our results indicate that it is difficult if not impossible to predict the pressure range at which normokinetic perfusion will be obtained

L E Carlgren & S Hagberg (Goteborg Sweden) *Cardiovascular infant surgery. Indications, methods and results*

At the Surgical Clinic of the Children's Hospital Goteborg 73 infants under 1 year of age have been operated upon for cardiovascular

Thirty-eight of the patients were male, and 22 female. Fifty-five cases could be classified as anal or rectal agenesis, 27 being of the latter type.

The results of treatment were excellent in 13, good in 10, satisfactory in 7, and poor in 6 of the surviving patients. No follow-up examination was made in respect of seven patients, and there were 17 deaths.

In 21 cases additional congenital anomalies complicated the situation. In 17, the other malformations were potentially lethal, and 13 of these cases terminated fatally. In 4 patients death was attributable to technical errors or failure to assess the condition adequately.

In 10 of the patients with imperforate anus the presence of Hirschsprung's disease was later confirmed or strongly suspected.

L Lindell & T Aalto (Turku, Finland) *Cine fluorography. Cine fluorographic study of patients with Hirschsprung's disease after operation*

A cine film was presented showing by fluorographic means the emptying of the rectum in one case operated on by the Soave technique and six by the Duhamel procedure. Some aspects of normal bowel and sphincter function were pointed out to aid recognition of the hypoganglionic segment, scar contracture of the sphincter and relaxation of the pelvic diaphragm in the cases concerned. Mention was also made of some difficulties and risks involved in the method.

H C Sommerchild & A Bjorkheim (Oslo, Norway) *Intestinal perforations as a complication in exchange transfusions in neonates*

Exchange transfusion through the umbilical vein is nowadays the standard treatment of neonatal hyperbilirubinaemia. However, the risk of intestinal perforation as a complication in this procedure is a new observation mentioned for the first time by Herman in 1965, but reported in a further 13 cases during November

1968. Most of the perforations have occurred in the colon.

The author presented another case, with a review of 20 cases from the literature. There was a survival rate of 75%, in contrast to 15% survival from spontaneous colonic perforations without previous exchange transfusion. This suggests that as regards pathogenesis the exchange transfusion cases differ from cases of spontaneous enteric perforation.

It was suggested that mechanical factors constitute the most important common denominator in cases following the exchange procedure. Stress was laid upon some observations which support this suggestion.

T Norman & H Otnes (Oslo, Norway) *Diffuse intestinal ganglioneuromatosis*

Two cases of diffuse intestinal ganglioneuromatosis are reported. In one patient, a 15-year-old boy, the lesion was associated with medullary carcinoma of the thyroid, severe diarrhoea and mucosal neuromas. Three previous reports have been published of patients with a similar syndrome. In all of them additional bilateral pheochromocytomas were found.

The second patient, a 3-year-old girl, exhibited not only diffuse ganglioneuromatosis but also diffuse intestinal lymphoid polyposis but with none of the other stigmata of the syndrome mentioned above.

U Schafer (Bremen, BRD) *Sphincter myectomy in Hirschsprung's disease*

Since 1951 315 cases of Hirschsprung's disease have been treated at the Children's Hospital, Bremen. In 277 cases intraabdominal anterior resection was performed. In 11 of these cases (4.5%) sphincter myectomy was performed as even three sphincter dilatations after the resection did not lead to regular evacuation of the bowel. The results after sphincter myectomy were satisfactory in 10 cases; the stubborn constipation was overcome completely.

In the light of experience it is possible that an equal number of myectomies will still be

isation and angiocardiography showed partial anomalous venous drainage from right lung to right atrium and an obliterated left main pulmonary artery but no septal defect. At the age of 7 years a second operation was performed during cardio-pulmonary bypass. The pulmonary veins were transposed to the left atrium. At follow up after 1 year the child's condition was quite satisfactory. The combination of anomalies seen in this case has to our knowledge not previously been reported.

2 Cyanosis at birth. Systolic and diastolic murmurs. At 3 weeks right middle lobe (RML) emphysema and clinical cardiac examination led to a diagnosis of pulmonary stenosis and ventricular septal defect. At 10 weeks bronchography performed on account of respiratory distress, increasing cyanosis and cyanotic spells showed a stenosed RML bronchus and cardiac catheterisation confirmed the clinical diagnosis. The stenosed bronchus was shown to be displaced by a prostenotic dilated pulmonary artery. A RM lobectomy was performed as an emergency operation. Follow up at 6 months of age was satisfactory.

3 Tachypnoea and cyanotic spells since birth. LUL emphysema. Lobectomy at 3 weeks of age on vital indications. Postoperatively RML emphysema and RUL atelectasis. RML bronchus possibly stenosed by aberrant artery. Bilobar emphysema has been established in this case.

It is of importance to diagnose the association of infantile lobar emphysema and a cardiovascular anomaly. In some cases the treatment of the cardiovascular condition may have a marked influence on the development of lobar emphysema and hence on the prognosis of the patient. The diagnostic armamentarium should include bronchography, cardiac catheterisation and angiocardiography.

G Semb & K V Hall (Oslo, Norway) *Waterston shunts*

Shunts from the systemic artery to the pulmonary artery with the technique described by

Waterston has great advantages especially in paediatric surgery where this is indicated. The operation was performed on 10 patients under 2 years of age. 5 cases of Fallot's tetralogy, 3 cases of pulmonary atresia and 2 cases of tricuspid atresia. The indications for operation were increasing cyanosis, cyanotic spells, pulmonary infections or progressive cardiac failure.

The operative method was described in detail with particular reference to the size of the shunt.

The clinical results were discussed and related to the objective improvement in hypoxia and polyglobulinaemia.

The postoperative complications and mortality and morbidity figures were presented.

FREE PAPERS

K V Parkkulanen (Helsinki, Finland) *Cine urethroscopy in boys*

A film was presented with the aim of demonstrating the modern possibilities of endoscopic filming in children and even in infants. Hitherto the interpretation of what is seen at endoscopy has been entirely dependent upon the subjective understanding and personal experience of the urologist who has had no opportunity for a subsequent detailed study of the appearances. Endoscopic filming allows examination of the wide variations observable in normal urethras and comparison of the findings with those in voiding cystography. At the same time documentation of normal and pathological conditions has become possible. Six cases were presented together with the respective voiding films.

R. Stenstrom & J. Elo (Helsinki, Finland) *Nephrobarinosis after micturition urethrocytography (UCGI) in children*

Among 1 600 UCG examinations of children made at the Aurora Hospital during the period 1957-1969, 3 cases of nephrobarinosis were found after urethrocytography with a sterile barium suspension. Nephrobarinosis must be

malformations since 1965. Of these, 25 have died.

Twenty cases of patent ductus arteriosus have been operated on, with only one death, which resulted from aspiration. This low mortality must be set in relation to the fact that the indications for operation were wider in those patients who did not exhibit any signs of concomitant cardiac malformation than in those who did. In 22 cases of pulmonary hyperfusion due to other left to right shunts, pulmonary banding was carried out, with 11 deaths. In these cases the lesions were extremely severe and without surgery the mortality would probably have approached 100 per cent.

Ten patients were operated upon for coarctation of the aorta, and 3 of these died. Of the survivors, 3 still have a high pressure gradient between the arms and legs and will probably have to undergo re-operation in the future. The remaining 4 patients are alive and in good condition. Seven cases of pulmonary atresia or severe stenosis with intact ventricular septum were treated, with four operative deaths. We have applied both open and closed methods, with a dilator.

Five patients with transposition of the large vessels were operated upon by the Blalock-Hanlon method. One of these died immediately after the operation, and another during a later operation. The remaining 3 patients are alive and in fairly good condition. Finally a shunt operation was performed in 9 cases with cyanotic heart disease and pulmonary hypofusion with four deaths.

O Viikari (Turku, Finland) Cardiac surgery in infants at Turku University Hospital

Since 1962 48 patients under 2 years of age have been referred to the paediatric surgical services of Turku University Hospital for refractory cardiac failure. In every case, the condition of the patient was deteriorating despite adequate medical treatment and the prognosis was considered extremely poor. In many of the cases, life could be maintained only by inten-

sive care and/or respiration treatment before surgery. Of the total series of 48, 23 were restored to normal health by corrective operation. Seventeen were definitely improved by palliative operation. In 8 patients surgery did not alleviate the cardiac distress and 6 of them died. A patent ductus arteriosus was the main reason for the cardiac failure in 17 patients. On follow up examination the cardiac status was virtually normal in all of them.

Coarctation of the aorta was the reason for the heart failure in 9 patients. Five were cured, three recoarctations were observed after initial improvement and 1 patient died.

Pulmonary hyperaemia, with VSD as the underlying lesion was relieved by pulmonary banding in 10 patients. One case complicated by coarctation proved fatal. All the others showed noteworthy postoperative improvement. Electro-encephalographic monitoring was used as an index of adequate brain oxygenation to determine the tightness of the band.

Pulmonary hyperaemia due to Fallot's pentalogy was palliated by aorto-pulmonary shunt in one of them and by right subclavio pulmonary shunt in 3 patients. All of them improved.

Other anomalies consisted of truncus arteriosus communis (3), aorto-pulmonary window (1), tricuspid atresia (1), ectopia cordis (1) and vascular ring (1). Of these only the last was cured by surgery and 4 patients died.

G Semb, T Kluge, S Sjøstrand & E Ek (Oslo, Norway) Infantile lobar emphysema. Three cases with associated congenital cardiovascular anomalies

Infantile lobar emphysema is a dangerous disease and therefore of importance. The clinical picture and radiographic appearance are characteristic. The three cases with associated congenital cardiovascular anomalies were:

1. LUL emphysema, LLL atelectasis, rotation, dextrocardia and right sided aortic arches. At the age of 3 years LU lobectomy was performed. Pulmonary infections continued and a systolic murmur was detected. Cardiac cathete-

isation and angiocardiology showed partial anomalous venous drainage from right lung to right atrium and an obliterated left main pulmonary artery but no septal defect. At the age of 7 years a second operation was performed during cardio-pulmonary bypass. The pulmonary veins were transposed to the left atrium. At follow up after 1 year the child's condition was quite satisfactory. The combination of anomalies seen in this case has to our knowledge not previously been reported.

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FREE PAPERS

A. V. Parkkinen (Helsinki, Finland) *Circulo-urethroscopy in boys*

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R. Sennstrom & J. Elo (Helsinki, Finland) *Nephrobarinosis after micturition urethrocytography (UCG) in children*

Among 1 600 UCG examinations of children made at the Aurora Hospital during the period 1957-1969, 3 cases of nephrobarinosis were found after urethrocytography with a sterile barium suspension. Nephrobarinosis must be

regarded as a relatively grave complication, for it can lead to permanent damage to the kidney. Persistent proteinuria and haematuria may result. In one of our cases acquired hypoplasia of the corresponding kidney was found 1 year after the examination; in this case massive infection was apparent just after the UCG. The authors recommend that the use of suspensions of barium sulphate for UCG be abandoned.

E Edelman & P Vilkkı (Turku, Finland)
Pressure measurement in the urinary tract

In the Department of Paediatric Surgery of the University Hospital, Turku, pressure measurements have been made in the distal ureters and bladders of children with radiologically demonstrated vesico-ureteric reflux, and in non-refluxing ureters and bladders of children examined for other urological reasons. The children were all free from urinary infection at the time of examination. The pressure measurements were made during normal cystoscopy by application of the same standard method. In dwelling catheters with an external diameter of 1 mm have been employed and the pressures recorded with an Electromanometer EM 31 manufactured by Elema-Schonander. The pressures in the distal ureter and in the bladder were recorded simultaneously with the degree of filling of the bladder with saline solution.

The series examined consisted of 23 ureters. With the bladder empty the functional efficiency of the ureter, which is reflected in pressure alterations, seems to be inferior in the refluxing ureters to that of the non-refluxing ureters. The functional efficiency in the refluxing ureters also seems to be impaired while the bladder is being filled, although this is not normally the case in non-refluxing ureters.

T Parvinen, O Nielsen & R Nielsen (Turku, Finland)
Ectopia vesicae and duplication of the bladder in the same patients

A presentation was made of 2 cases of duplication of the urinary bladder combined with

extrophy of the ventral compartment, along with a short anatomical and embryological survey.

O Lindfors & R Anttila (Helsinki, Finland)
Experience with seven renal homotransplantations in children

Although kidney transplantation is the accepted and established therapy in the routine treatment of renal failure, the literature on paediatric kidney transplantations is sparse. The inevitable immunosuppressive treatment may be expected to retard the normal development of the child. The prognosis is thought to be poor. Since 1967, the kidney transplantation team at the University of Helsinki has performed kidney homotransplantations on seven children of ages ranging from 9 to 15 years. All the patients had severe uraemia, including hypertension and retinal changes. All the donors were parents. Immunosuppression was maintained with prednisone 0.1–0.3 mg/kg/day and azathioprine 1.0–2.0 mg/kg/day. The follow-up has now covered a period of 14–42 months (in 4 patients, more than 2 years). One month after the transplantation 1 patient died of gastrointestinal haemorrhages and peritonitis. His kidney function, however, was quite normal. Four patients have had only one rejection, usually 3–6 weeks after transplantation. Thus only 2 children have had more than one rejection. In instances of rejection the therapy applied comprised not only higher doses of prednisone and azathioprine but also local irradiation of the graft (total dose 600–1 000 R) and Actinomycin C (200 µg) every other day until the rejection was halted. Other complications observed were the nephrotic syndrome in one case, urinary complications in 2 patients and encephalitis in one. Four patients now have normal blood pressure and the other two moderate hypertension. Serum creatinine is normal and retinal changes have almost regressed. Slight osteoporosis has been observed. The skeletal age is somewhat lower than normal, but in 1 patient is 2 years in advance of the chrono-

logical age. Growth in height has been minimal. The gross appearance is more or less Cushingoid except in one child who had better compatibility and has needed less suppression therapy. The surviving patients (6/7) all attend school, move actively and are no longer bound to dialysis centres. Most of the routine checks are made at local hospitals. It is too early to draw conclusions about the future of children who have undergone kidney transplantation but the major problem seems to arise from the side effects of immuno-suppression. In future even more attention should be paid to optimal immunological matching.

B. H. Thorasson & M. M. Ravitch (Illinois USA): Foetal surgery in the rabbit

The film shown demonstrated the technique of endotracheal intubation, laparotomy and hysterotomy in a pregnant doe. Further foetal ureteral ligation and small bowel transection on the 25th day of gestation were shown. The form was carried out entirely in utero and the latter by temporary extrusion of the foetus in its intact membranes through the hysterotomy.

The technique was developed during the course of about 400 operations on foetuses with the aim of elucidating the foetal response to trauma and the pathogenesis of polycystic kidney and meconium peritonitis. The foetal survival rate was about 60%.

J. Viljanto & S. M. Vutanen (Turku Finland): Thermographic observations of wound healing in children

The skin temperature around an inguinal or abdominal wound was studied by means of thermovision in 24 children under 13 years of age and in 5 adults aged 18-67 years. Most of them had undergone operation for inguinal hernia or for incomplete descent of the testis. Two patients with pyloric stenosis and two with Hirschsprung's disease were included. The wounds were covered with Nobecutan® film

which being in direct contact with the skin did not prevent its thermal radiation.

It was observed that in the group of the infants under 1 year of age the wound was visible as a cool line for about 1 week. The surrounding skin was warmer for 3-5 days postoperatively. In the group aged 2-4 years the cool wound line was visible for 10-15 days and the warm area around it for about 3 weeks. However, the monophasic thermal curve of the younger patients was now replaced by one of biphasic form which attained the first maximum on the 5th day and the second on the 12th-14th day. In children aged 8-12 years the biphasic nature of the increasing skin temperature around the wound was even more pronounced. The peak values were attained during the first 10 days and again during the 3rd or 4th week. The wound was seen as a cool line for 3 weeks.

The first phase of increased skin temperature around the wound might be attributable to vasodilatation during the period of so-called post-traumatic inflammation. The second phase could be regarded as an expression of the high metabolic activity of connective tissue cells although many other causes might be suggested.

R. Bjørdal (Oslo, Norway): Septic candida albicans infection

During the period January 1969 to February 1970 septic *Candida albicans* infection was observed in 15 children whose ages ranged from 2 weeks to 12 years in the D partment of Paediatrics at Rikshospitalet. Eleven of these children had been admitted to the section for paediatric surgery.

Thirteen of the patients succumbed and in these the septic *Candida* infection was disclosed at autopsy. In 2 children, the diagnosis was made intra vitam. Fungizone treatment was begun and the patients survived.

Common denominators in these cases were bacterial infection, deterioration, treatment with antibiotics, parenteral alimentation.

E S Heikkinen & M Sulamaa (Helsinki, Finland) *Habitual dislocation of the hip*

A report was presented on two children, a boy and a girl, with habitual dislocation of the hip. They were examined and treated at the Children's Hospital, University of Helsinki. In the boy, both hips were affected, and in the girl, the right one.

The main symptom was snapping or popping noises of the hip, which in both cases started at the age of 1.5 years. In infancy, the hips were clinically normal. The children had no trauma, no dysplasia in the hip joints, no capsular defect, and no neurogenic disorders. The boy and the girl could each dislocate and reduce the hip at will. The provocation test was positive at the age of 5 years in both patients. At that time, the posterior dislocation of the femoral head could be seen, felt, heard, and confirmed by X-ray examination. Initially the boy was treated unsuccessfully with an abduction splint. After 6 months this treatment was discontinued, but the boy recovered spontaneously at the age of 8 years. He is now 16 years old, and completely free from symptoms

arising from the snapping hip; the girl had muscular atrophy in her right thigh, with occasional pain, and limped slightly. In view of the progression of these symptoms, it was decided to operate. Now 1 year after the operation the joint is stable, and its movements are normal.

S Einola, P Viikari, E Melartin & B Thomasson (Turku, Finland) *Luxatio congenita patellae*

A film was shown to present a case of congenital lateral dislocation of the patella in an 11-year-old boy with progressively worsening gait and flexion contracture of the knee. The correction of this condition by means of retinacular release and ligamentoplasty was demonstrated. The report included a follow-up examination, 8 to 15 months after surgery of 5 similar patients, 3 to 16 years old.

G R Wallgren
Aurora Hospital
Helsinki
Finland

BOOK REVIEWS

G Debrun & C Gasquet *Explorations vasculaires des tumeurs abdominales de l'enfant* L'Expansion Paris 19 0 92 pp illus F 40

This book belongs to a series of monographs published by the journal *Annales d' Radiologie*. Its first part (written by Debrun) reports the experience with abdominal angiography in 26 cases of benign tumors and 77 cases of malignant neoplasms most of which originated in the retroperitoneal ganglia the kidney the adrenal or the liver. The findings are exemplified by instructive reproductions. The second part (by Gasquet) is concerned with lymphography of 105 cases of malignant disease and is equally well illustrated. The majority of these tumors were neuroblastomas or renal or ovarian neoplasms.

Though lymphography and abdominal angiography are now widely used in the exploration of malignant disease in adults experience with these diagnostic methods in infancy and childhood is still limited. The book is therefore a useful contribution to the literature in an important and expanding field of pediatric radiology.

G Theander

Nancie R Finnie *Börn med cerebral parese* Borgens Forlag Copenhagen 1970 192 pp illus D kr 36 00

Nancie R Finnie is a physiotherapist working at the Bobath Institute. She has vast experience in the treatment of cerebral palsied children according to the Bobath principles. The book is intended to be a guidance for both parents physiotherapists and other therapists.

This book supplies a long felt want and it is very valuable that the book is now translated into Danish by Karen Bysted a physiotherapist with many years experience in the treatment of CP children. The translation is well adapted to Scandinavian circumstances and made with great understanding of the authors' intentions.

The book is illustrated with instructive drawings which make it easy to read and thus avoiding too difficult explanations and medical terms. There are separate chapters about bathing feeding and other daily activities and also suggestions about different technical aids.

The importance of cooperation between parents and therapists is emphasized. To avoid confusion the parents must have the opportunity to discuss appropriate sections of the book with therapists. This is a valuable book giving a better understanding of CP children's problems and good advices for their treatment.

Ing id Bjerre

W Ludwig *Das Rechts Links Problem in Tierreich und beim Menschen* Mit einem Anhang "Rechts Links-Merkmale der Pflanzen" Springer Verlag Berlin, Heidelberg and New York 1970 496 pp illus.

This work was originally published as volume 27 of *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere* 1932 and the present edition is an unamended reprint. The work is thus almost 40 years old but nevertheless it gives an excellent summary of the problems concerned with the asymmetric development of a great number of animals including man. The work is divided into three parts: (1) An introduction of 29 pages being mainly a matter of terminological contents. (2) A thorough account of the asymmetry within the animal kingdom and particularly in relation to man. (3) A general discussion on the problems. Some of the illustrations of asymmetrical development in the lower invertebrates appear somewhat problematic but they have significance in general and do not detract from the value of the work. The greatest value for physicians will probably be found in the paragraphs concerning left handedness and other consequences from inverse asymmetric development in man. Unfortunately the subject on psychological aspects (in ferority complexes etc.) has only been briefly touched on.

G Mandahl Barth

R Burkhardt *Farbatlas der klinischen Histopathologie von Knochenmark und Knochen* Springer Verlag Berlin Heidelberg and New York 1970 115 pp illus DM 248 —

This beautiful and expensive atlas of bone marrow histopathology is based upon the experience gained from 2 200 biopsies. Although the size of the author's material does not seem impressive the high quality of the sections produced in his laboratory is obvious from the 700 microphotographs in colour which form the bulk of the volume. An introductory chapter gives a detailed description of biopsy technique and the method used for cutting and staining the undecalcified bone marrow sections.

Part of the atlas clearly suffers from superfluity of illustrations. Several microphotographs are thus devoted to vascular changes which certainly look the same in the bone marrow as elsewhere in the body. For instance a cross section of a muscular artery whether dilated or contracted is hardly warranted in an atlas of bone marrow histopathology. Three microphotographs serve to illustrate the well known appearance of vascular amyloidosis. One whole

page—ten microphotographs—claims to show various phases of thrombus formation

The various aspects of genuine bone marrow pathology i.e. disturbances of the hematopoietic and reticuloendothelial systems which naturally occupy the greater part of the atlas are outlined with considerably more care and accuracy. However the general question might be raised whether this type of textbook (or atlas) should be written by a pathologist with a particular interest of hematology rather than by a clinical hematologist interested in histopathology. It is the reviewer's biased opinion that bone marrow histopathology like dermatohistopathology and any other special field of pathology is best managed by individuals with a broad experience of general pathology.

Bengt Robertson

F. C. Fraser & V. A. McKusick (eds) *Congenital malformations*. Proceedings of the Third International Conference. The Hague, September 1969. *Excerpta Medica*, Amsterdam, 1970. 466 pp. illus. US\$ 27.50.

The science of congenital malformations/teratology has been the subject of three international congresses—the first was held in 1960, the second in 1963 and the third in 1969. The reports of all three congresses have been published in the *Excerpta Medica International Congress Series* and give admirable summaries of the development of interest in and knowledge of this subject. The third volume contains a number of very good review lectures, most however of a fairly superficial nature. Some basic fields related to teratology have been summed up under the heading of *Developmental Biology 1969*. Little really new data are given but the summaries presented may be of value for the non-specialist to keep track of what happens in these rapidly expanding fields. Other reviews deal with the uterine milieu and the early embryo and the remaining ones are dedicated to human teratology. Besides such review articles the chairmen of a number of parallel discussion and paper sessions have given short summaries of the original data presented or the discussions held.

I found these chapters to be of limited value because of their superficiality.

This volume is good reading for anyone who wants to make himself acquainted with the present state of the international research dealing with malformations. It should be of interest not only to the embryologist and teratologist but also and perhaps still more to the pediatrician and other specialists who want to be informed on basic research in this important field.

Bengt Kallen

O. Gross & R. Ortmann *Grundriss der Entwicklungsgeschichte des Menschen*. 7th Ed. Springer Verlag, Berlin Heidelberg and New York, 1970. 207 pp. illus. DM 28.—

Knowledge about human embryology is an important basis for the understanding of both adult anatomy and the origin of anatomical deviations and malformations. Human embryology is therefore an essential part of the medical curriculum. There are many textbooks available, varying in both size and quality. The 7th revised edition of a classical German textbook has now been published. It describes the formal genesis of the human body from fertilization to birth and is well illustrated with numerous microphotographs, diagrams and reconstructions. The descriptions are relatively brief and give the bare basic knowledge for the student—in some sections, e.g. the clinically important section of urogenital tract development. The text is not quite up-to-date.

The author has tried to introduce some more modern aspects on development by brief references to cytogenetics, experimental embryology, teratology etc. These discussions are very short. As the book is especially suitable for medical students, more information on the common malformations and their embryological origin would have been welcome if only to increase the motivation for the students to study embryology. On the other hand, the final chapter on "Geburtszustand" with a summary of the Poimann ideas could from this aspect well be omitted.

Bengt Kallen

ANNOUNCEMENT

The 3rd European Congress of Pediatric Neurosurgery takes place in Göttingen, West Germany, from 21st to 23rd September 1972. For information please

address Prof. Dr. med. K. A. Bushe, Direktor d. Neurochirurgischen Klinik der Universität Göttingen, 3400 Göttingen (West Germany), Gosslerstraße 10.

EFFECTS OF DIET ON FATTY ACID COMPOSITION OF PLASMA AND RED CELL PHOSPHOGLYCERIDES IN THREE MONTH OLD INFANTS

RAGNAR OLEGARD and LARS SVENNERHOLM

*From the Department of Paediatrics and the Department of Neurochemistry
Psychiatric Research Centre University of Göteborg Göteborg Sweden*

It has been shown by Anild Hansen and his school (6-8) that insufficient dietary linoleic acid will result in clinically manifest essential fatty acid deficiency in the human infant. Hansen et al (8) also found that the blood serum levels of polyunsaturated fatty acids reflected the dietary intake of linoleate remarkably well. From parallel animal experiments headed by Ralph Holman methods were developed which were applied in the estimation of the linoleate requirements of human infants (10). The estimated minimum requirement of linoleate was approximately 14% of total calories.

Infants fed cow's milk formulas consisting of cow's milk diluted 1:1 with water and containing added sucrose may show biochemical signs of insufficient dietary linoleate (11-16). But during the last decade industrially manufactured cow's milk formulas used instead of breast milk in Scandinavia and several other Western countries contain supplementary linoleate. In Sweden the linoleate concentration used is approximately the same as that in breast milk or at least 4 calories. Only relatively few studies are available on the effect of linoleate fortified formulas on the blood fatty acid pattern. Compared with the level in breast fed infants Hansen et al (7) found that a cow's milk formula with 7.2 calories linoleate enhanced the plasma lipid linoleic acid but suppressed arachidonic acid while Mendy et al

(16) reported that a formula with 5.5 calories dietary linoleate raised both of the two acids in plasma lipids. The observation periods were short in these studies particularly in the latter. The controversial results of the effect of the long term use of a linoleate-enriched formula prompted us to undertake this study. The dietary period selected was the first 3 months of life i.e. a period long enough to reduce the effect of the composition of the fat depots at birth and short enough to preclude any effect of dietary supplements to the milk diet.

MATERIAL AND METHODS

Clinical material

Formula fed infants Nine apparently healthy and normally developed infants in an infant nursery (My rormas spädbarnshem Göteborg) were arbitrarily selected for the study. The infants were fed formulas based on skimmed cow's milk fortified with vegetable fat (Findus Tillägg Findus AB). The composition of the formula is given in Table I. Fruit juices had been added to the diet from 8 weeks of age. One infant was found to have hypercholesterolemia and an abnormal cholesterol:phospholipid ratio and was therefore excluded. Thus left eight infants for the present study.

Breast fed infants One healthy infant in the same infant nursery as above was given pooled breast milk from the Breast Milk Central Göteborg. Nine healthy infants were arbitrarily selected in a Welfare Centre for infants and children in Göteborg. These infants received breast milk from their own mothers and an addition of fruit juices from 6 to 8 weeks of age.

page—ten microphotographs—claims to show various phases of thrombus formation

The various aspects of genuine bone marrow pathology i.e. disturbances of the hemopoietic and reticuloendothelial systems which naturally occupy the greater part of the atlas are outlined with considerably more care and accuracy. However the general question might be raised whether this type of textbook (or atlas) should be written by a pathologist with a particular interest of hematology rather than by a clinical hematologist interested in histopathology. It is the reviewer's biased opinion that bone marrow histopathology, like dermatohistopathology and any other special field of pathology, is best managed by individuals with a broad experience of general pathology.

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Table 3 Mean concentration of plasma lipids of newborn infants 3 month old infants and adults mg/100 ml

	Newborn infants (17) (n = 10)		3-month-old infants				Normal adults (22) (16-38 years)			
			Breast fed (n = 10)		Formula fed (n = 8)		Women (n = 28)		Men (n = 62)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Total lipids	257 ^a	59	609 ^a	91	637 ^a	82	648 ^b	114	610 ^b	120
Triglycerides	39	70	168	72	211	64	88		84	34
Cholesterol	66	18	153	29	147	17	185	38	192	57
Phospholipids	113	24	101	38	194	25	232	44	208	34
Cholesterol/phospho- lipid ratio	0.59	0.07	0.77	0.08	0.76	0.07	0.80		0.95	

Calculated ^a Gravimetric determination

infants was still larger in red cell lecithin than in plasma phosphoglycerides

The concentration of the fatty acids of the linolenic acid series in the formula fed infants was half of that in the breast fed infants in whom it was in the range for normal Swedish adults (2-17). Among the individual fatty acids of the linolenic acid series the difference

was largest for the most poly unsaturated acid of the series 22:6 (n-3)

Fatty acid composition of red blood cell cephalins

The concentration of polyenoic acids was significantly higher in the formula fed infants (Table 5). The sum of fatty acids of the lino-

Table 4 Fatty acid composition of plasma phosphoglycerides and of red blood cell lecithin in breast fed and formula fed 3 month old infants

Fatty acid	Plasma phosphoglycerides					Difference Formula fed Breast fed Mean	Red blood cell lecithin				
	Breast fed (n = 10)		Formula fed (n = 8)		Breast fed (n = 7)		Formula fed (n = 8)		Difference Formula fed Breast fed Mean		
	Mean	S D	Mean	S D	Mean		S D	Mean		S D	
16 0	28.5 ^a	3.64	24.5	2.09	-4.0 ^b	34.6	1.11	31.0	4.41	-3.6	^b
16 1	1.9	0.82	1.50	0.35	-0.4	2.2	0.34	1.7	0.32	-0.5	
18 0	18.2	0.72	14.0	2.27	-4.2	14.8	0.78	11.5	1.55	-3.5	
18 1	14.0	0.62	16.8	3.21	+2.8	21.6	1.10	21.8	1.28	+0.2	—
18 2 (n-6)	20.0	1.13	27.3	0.96	+7.3	13.2	1.46	22.2	1.90	+9.0	
18 3 (n-3)	0.7	0.13	0.7	0.16	±0	1.0	0.24	0.8	0.12	-0.2	
+20 1											
20 3 (n-3)	traces		traces			traces		traces			
20 3 (n-6)	2.5	0.43	3.5	0.39	+1.0	1.8	0.31	2.2	0.28	+0.4	**
20 4 (n-6)	8.7	1.64	7.7	1.34	-1.0	7.4	1.57	6.2	1.23	-1.2	—
20 5 (n-3)	0.5	0.18	0.3	0.26	-0.2	0.3	0.08	traces		-0.3	
2 4 (n-6)	0.3	0.11	0.9	0.14	+0.6	0.5	0.18	0.8	0.27	+0.3	*
22 5 (n-6)	0.2	0.05	0.9	0.18	+0.7	0.1	0.04	0.5	0.17	+0.4	
22 5 (n-3)	0.7	0.29	0.3	0.17	-0.4	0.4	0.06	0.2	0.09	-0.2	
22 6 (n-3)	3.8	1.36	1.4	0.26	-2.4	2.1	0.67	1.0	0.36	-1.1	**
18-22 (n-6)	31.7	2.09	40.4	1.32	+8.7	22.9	1.38	32.1	1.94	+9.2	
18-22 (n-3)	5.6	1.13	2.6	0.17	-3.0	3.9	0.67	2.3	0.48	-1.8	**
(n-6) + (n-3)	37.2	3.16	43.0	1.63	+5.8	26.8	1.75	34.1	0.97	+7.3	*

^a Values are weight percentages of methyl esters^b Significance levels: -0.05 level -0.01 level -0.001 level

Table 1 Composition of breast milk and of the cow's milk formula

	Breast milk	Formula
Calories/l	690 ^a	670 ^b
Percentage of calories		
Protein	7 ^a	14 ^b
Carbohydrates	42 ^a	53 ^b
Fat	51 ^a	33 ^b
Saturated fatty acids	21 ^c	11 ^c
Monounsaturated fatty acids	20 ^c	12 ^c
Linoleic acid	3.5 ^c	5.4 ^c
Linolenic acid	1.2 ^c	0.3 ^c
Arachidonic acid	0.5 ^c	0.2 ^c

^a Data from I G Macy & H J Kelly (15)^b Analysed by Findus AB Laboratories Bjuv Sweden^c Own GLC analyses

The concentrations of linoleic, linolenic and arachidonic acids from a pooled sample of breast milk are given in Table 1.

Age and weight on the day of blood sampling, birth weight and gestation age are given in Table 2. The pregnancies and deliveries of the mothers had been uneventful. Neonatal life had also been uncomplicated. No infant had any known illness at the time of blood sampling.

Blood sampling

Blood samples obtained from the internal jugular vein or as freely flowing blood from an incision in the sole of the foot were collected directly in heparinized test tubes. Plasma was separated from blood cells generally within 4 hours and afterwards treated in the way described previously (17).

Chemical methods

The preparation of lipid extract, quantitative assay for lipid P, cholesterol and triglycerides, separation of lipids by column chromatography with silicic acid, methanolysis of phosphoglycerides and GLC of methyl esters were performed exactly as described in a previous paper (17).

Statistical methods

The significances of the differences in fatty acid concentrations and concentrations of main lipid classes between the two groups were tested by direct *t* test of the means.

RESULTS

Concentration of main lipid classes in plasma

The mean values and standard deviations found for the two groups of infants are given in Table 3. The total lipids were calculated as the sum

of the various lipid classes. The cholesterol figure was multiplied by 1.44 to include the fatty acids in cholesterol esters. For free fatty acids, a figure of 20 mg/100 ml was added.

No significant difference in the concentration of any lipid class was found between the breast fed and the formula fed infants. The mean triglyceride concentration was somewhat higher in formula fed infants.

Cholesterol and phospholipid levels had almost reached normal adult levels but the triglyceride level was about twice as high as the adult level.

The cholesterol/phospholipid ratio was close to that for adult women (Table 3).

Fatty acid composition of plasma phosphoglycerides and red blood cell lecithin

The plasma concentration of polyenoic acids was significantly higher in the formula fed group while the concentration of saturated fatty acids was higher in the other group (Table 4). The concentration of linoleic acid and the sum of all fatty acids of the linoleic acid series was about 30% lower in the breast fed infants. But there was no significant difference in the concentration of arachidonic acid between the two groups.

The red cell lecithin content of polyenoic acid was also significantly higher in the formula fed infants. The difference in the concentrations of fatty acids of the linoleic acid series between the breast fed and the formula fed in

Table 2 Age and weight at blood sampling, birth weight and gestational age

	Mean	Range
<i>Breast fed infants (n = 10)</i>		
Age	96 days	82-116 days
Weight	5 670 g	4 650-6 600 g
Birth weight	3 620 g	2 750-4 180 g
Gestational age	40.0 weeks	38-43 weeks
<i>Formula fed infants (n = 8)</i>		
Age	100 days	88-117 days
Weight	5 260 g	4 500-5 880 g
Birth weight	3 440 g	2 750-4 180 g
Gestational age	40.0 weeks	38-41 weeks

hydrate/fat ratio in the formula than in the breast milk (1). At birth the triglyceride plasma concentration is very low and at one year of age the triglyceride level is the same as in adulthood (Table 3). The 100% higher plasma triglyceride concentration of our 3 month old infants coincided in time with the very high glucose tolerance found by v Euler et al (3) in this age group. These authors suggested "a relative hyperinsulinism" at this age which could also explain the hypertriglyceridemia in the infants since a high correlation has been demonstrated between the insulin response to high carbohydrate diet and the degree of hypertriglyceridemia (20) in adults.

Fatty acid composition

The fatty acid composition of plasma phosphoglycerides and red blood cell lecithin of the 3 month old infants had changed from the newborn to the adult pattern in both groups of infants. Linoleic acid (18:2 (n-6)) had increased and arachidonic acid (20:4 (n-6)) had diminished. The two groups did not differ significantly from one another in concentration of arachidonic acid in lecithin which was within the range for the adult Swedish population (2, 17).

There were two major differences in the fatty acid composition of plasma and red cell lecithin between breast fed and formula fed infants (Table 4). The sum of the polyenoic fatty acids was smaller in breast than in formula-fed infants and the ratio of the fatty acids of the linolenic acid to the linoleic acid series was lower in the formula than in the breast fed infants.

The latter difference was closely related to the dietary ratio of linolenate to linoleate (Table 2). It has earlier been amply demonstrated that the dietary ratio between these two fatty acids strongly influences the ratio of the fatty acids of the linoleic acid and linolenic acid series in total lipids of blood and various organs (9) and in the phosphoglycerides of brain and liver (5, 25). A low dietary linolenate/linoleate ratio

will lead to an increase of the fatty acids of the linoleic acid series particularly of 22:5 (n-6) (25). It is evident from Table 4 which gives the fatty acid composition of plasma and red cell lecithin that the amount of 22:5 (n-6) was many times larger in formula fed than breast fed infants while the concentration of the fatty acids of the linolenic acid series in formula fed infants was only half of that in the breast fed group. Recent dietary studies in the rat at this laboratory¹ have shown that the absolute amounts of linoleate and linolenate in the diets have but little effect on the ratio between the fatty acids of the linoleic and linolenic acid series of lecithin. This ratio was not significantly changed when the dietary concentration of the two acids was varied between 1 to 10 caloric % as long as the ratio between the two fatty acids was kept constant. On the other hand a moderate alteration of the dietary linolenate/linoleate ratio changed the ratio between the two fatty acid series in plasma lecithin. It is thus evident that a low plasma level of fatty acids belonging to the linolenic acid series cannot be used as a proof of lack of dietary linolenate.

In a previous study (17) it was shown that although the linoleic acid/arachidonic acid ratio of plasma and red blood cell lecithin in the newborns was the inverse of that in their mothers the concentration of the total polyenoic fatty acids was equal in both and fell within the same range as that in normal adults (2). In the present study the concentration of fatty acids of the linolenic acid series was higher in the plasma and red cell lecithins of breast fed than of formula fed infants but the concentration of the total polyenoic acids was significantly lower in the breast fed than in the formula fed infants and in newborns. The difference was confined to the fatty acids of the linoleic acid series. The concentration of the linoleic acid in blood lecithin was about 30% lower in breast fed than in formula fed infants the difference being somewhat larger

¹Olegrd, R. unpublished results

²Alling C, Karlsson I, Olegrd R & Svennerholm L. unpublished results

Table 5 *Fatty acid composition of red cell cephalins in breast fed and formula fed 3 month old infants*

Fatty acid	Breast fed (n=7)		Formula fed (n=8)		Difference Formula fed Breast fed Mean
	Mean	S D	Mean	S D	
16 0	13.7 ^a	1.25	11.2	1.59	-2.5 ^b **
16 1	1.6	0.50	0.8	0.14	-0.8 ***
18 0	25.1	1.94	19.2	0.87	-5.9 ***
18 1	16.2	1.26	17.3	0.57	+1.1 *
18 2 (n-6)	3.5	0.32	6.0	0.47	+2.5 ***
18 3 (n-3)					
+20 1	1.0	0.11	0.8	0.14	-0.2 *
20 3 (n-9)	traces		traces		
20 3 (n-6)	1.6	0.49	1.9	0.56	+0.3 -
20 4 (n-6)	22.6	2.45	24.6	1.81	+2.0 *
20 5 (n-3)	0.7	0.33	0.3	0.20	-0.4 **
22 4 (n-6)	3.7	0.69	8.9	1.73	+5.2 ***
22 5 (n-6)	0.7	0.24	2.3	0.47	+1.6 ***
22 5 (n-3)	2.0	0.33	1.6	0.64	-0.4 *
22 6 (n-3)	7.6	2.00	4.9	0.99	-2.7 **
18-22 (n-6)	32.5	2.69	43.6	3.04	+11.1 ***
18-22 (n-3)	11.3	2.48	7.5	1.54	-3.8 *
(n-6) + (n-3)	43.8	3.65	51.1	2.72	+7.3 *

^a ^b See text in Table 4

leic acid series also in red cell cephalins was 30% lower in the breast fed infants. The differences in concentrations were largest for linoleic acid (18 2 (n-6)) 22 4 (n-6) and 22 5 (n-6). The difference in arachidonic acid (20 4 (n-6)) concentration was smaller and significant only at the 0.05 level.

In conformity with the fatty acid patterns of plasma phosphoglycerides and red cell lecithin, the fatty acids of the linolenic acid series in red cell cephalins was 30% lower in the formula fed infants. The difference was largest for 22 6 (n-3). The concentration of the fatty acids of the linolenic acid series was in the same range for breast fed infants as for normal adults (2).

DISCUSSION

Concentration of main lipid classes in plasma

In adults a high intake of polyenoic acids has been shown to lower plasma cholesterol levels (12). Though breast milk contains about three times as much linoleate as cow's milk formula

Woodruff et al (26) found similar plasma cholesterol levels in 3 month old infants fed breast milk or the cow's milk formula. The results obtained in other studies (4, 7, 14, 18) of the effect of breast milk or cow's milk formulas on the plasma lipid composition during early infancy are difficult to evaluate because no information is given about the infants' ages at the time of blood sampling. There were also differences in μ g caloric intake, the type and the amount of carbohydrates and proteins as well as in the fat and linoleic acid contents which complicated comparisons of the results.

Pomeranze et al (19) showed that 50 g of corn oil added daily to an infant's evaporated milk formula significantly lowered serum cholesterol levels. Sweeney et al (24) found 30% lower values for plasma triglycerides, cholesterol and phospholipids and lower cholesterol/phospholipid ratios in 6 week old infants fed cow's milk formulas containing 10-15 caloric % linoleic acid than in age matched infants fed the same diet but with only 0.5 caloric % of linoleic acid. In a well controlled study by Lindquist & Malmcrona (13) the cholesterol level was shown to rise during the first one to two weeks after birth irrespective of the linoleic acid content of the diet. The cholesterol levels afterwards continued to rise in infants on a low-linoleic acid diet (cow's milk fat, about 1 caloric-% linoleic acid) but remained constant in the infants on a high linoleic acid diet (corn oil about 18 caloric % linoleic acid).

We could not find any difference in total lipid, cholesterol or phospholipid plasma concentration between the breast fed and the formula fed infants. As in previous studies moderate changes in the dietary polyunsaturated fatty acids did not appear to have any significant effect on the plasma lipid levels.

Although the difference in triglyceride concentration in the present study was not significant at the 0.05 level, the mean triglyceride concentration was 25% higher in formula fed than in breast fed infants. This difference might perhaps be explained by a higher carbo-

hydrate/fat ratio in the formula than in the breast milk (1). At birth the triglyceride plasma concentration is very low and at one year of age the triglyceride level¹ is the same as in adulthood (Table 3). The 100% higher plasma triglyceride concentration of our 3 month-old infants coincided in time with the very high glucose tolerance found by v Euler et al (3) in this age group. These authors suggested a relative hyperinsulinism at this age which could also explain the hypertriglyceridemia in the infants since a high correlation has been demonstrated between the insulin response to high carbohydrate diet and the degree of hypertriglyceridemia (20) in adults.

Fatty acid composition

The fatty acid composition of plasma phosphoglycerides and red blood cell lecithin of the 3 month old infants had changed from the newborn to the adult pattern in both groups of infants. Linoleic acid (18:2 (n-6)) had increased and arachidonic acid (20:4 (n-6)) had diminished. The two groups did not differ significantly from one another in concentration of arachidonic acid in lecithin which was within the range for the adult Swedish population (2, 17).

There were two major differences in the fatty acid composition of plasma and red cell lecithin between breast fed and formula fed infants (Table 4). The sum of the polyenoic fatty acids was smaller in breast than in formula fed infants and the ratio of the fatty acids of the linolenic acid to the linoleic acid series was lower in the formula than in the breast fed infants.

The latter difference was closely related to the dietary ratio of linolenate to linoleate (Table 2). It has earlier been amply demonstrated that the dietary ratio between these two fatty acids strongly influences the ratio of the fatty acids of the linoleic acid and linolenic acid series in total lipids of blood and various organs (9) and in the phosphoglycerides of brain and liver (5, 25). A low dietary linolenate/linoleate ratio

will lead to an increase of the fatty acids of the linoleic acid series particularly of 22:5 (n-6) (25). It is evident from Table 4 which gives the fatty acid composition of plasma and red cell lecithin that the amount of 22:5 (n-6) was many times larger in formula fed than breast fed infants while the concentration of the fatty acids of the linolenic acid series in formula fed infants was only half of that in the breast fed group. Recent dietary studies in the rat at this laboratory¹ have shown that the absolute amounts of linoleate and linolenate in the diets have but little effect on the ratio between the fatty acids of the linoleic and linolenic acid series of lecithin. This ratio was not significantly changed when the dietary concentration of the two acids was varied between 1 to 10 caloric % as long as the ratio between the two fatty acids was kept constant. On the other hand a moderate alteration of the dietary linolenate/linoleate ratio changed the ratio between the two fatty acid series in plasma lecithin. It is thus evident that a low plasma level of fatty acids belonging to the linolenic acid series cannot be used as a proof of lack of dietary linolenate.

In a previous study (17) it was shown that although the linoleic acid/arachidonic acid ratio of plasma and red blood cell lecithin in the newborns was the inverse of that in their mothers the concentration of the total polyenoic fatty acids was equal in both and fell within the same range as that in normal adults (2). In the present study the concentration of fatty acids of the linolenic acid series was higher in the plasma and red cell lecithins of breast-fed than of formula fed infants but the concentration of the total polyenoic acids was significantly lower in the breast fed than in the formula fed infants and in newborns. The difference was confined to the fatty acids of the linoleic acid series. The concentration of the linoleic acid in blood lecithin was about 30% lower in breast fed than in formula fed infants the difference being somewhat larger

¹Olegård ■ unpublished results

¹Alling C Karlsson I Olegård R & Svennerholm L, unpublished results

in the red cells than in the plasma. Also, in other members of the linoleic acid series, namely 22:4 ($n-6$), was significantly lower in the blood phosphoglycerides of breast fed infants. The absolute difference in the lecithin fraction was rather small, but was large in the blood cell cephalins.

The present findings raise the question whether the low concentration of fatty acids of the linoleic acid series and the total polyenoic acids in the blood phosphoglycerides are signs of deficient dietary linoleic acid in the breast-fed infant. It is widely accepted that a deficiency of essential fatty acids is reflected in an elevation of 20:3 ($n-9$) but this has not been proved in humans. There were only traces of this acid in plasma and red cell phosphoglycerides of the breast fed infants. We have previously stressed the importance of the homeostatic mechanism by which the level of polyenoic fatty acids of the phosphoglycerides is kept constant in the blood (17) and in the brain (23). The low concentration of total polyenoic acids in the blood phosphoglycerides suggests that this homeostatic mechanism is not fully developed or that the dietary intake of linoleic acid is insufficient in the breast fed infant during the first months of life.

SUMMARY

Concentrations of the major lipid classes in plasma and the blood phosphoglyceride fatty acid patterns were measured in 3 month old infants fed breast milk or an industrial manufactured formula. The levels of cholesterol and phosphoglycerides were in the normal range for young adults, but the triglyceride concentration was twice as high as in adults. There were no significant differences in the levels of the plasma lipids between the two groups but the mean of the triglycerides was 25% higher in the formula fed group.

The plasma and red cell phosphoglycerides had already assumed a fatty acid pattern of adult type. The concentration of the total polyenoic acids was significantly lower in the

breast fed group the difference being entirely confined to the fatty acids of the linoleic acid series. Linoleic acid was 27% and 40%, respectively, lower in plasma phosphoglycerides and red cell lecithin of breast fed than of formula fed infants, but arachidonic acid did not show any significant difference in the two groups. The concentration of the fatty acids of the linolenic acid series was higher in the breast fed infants. The blood lecithin ratio between the fatty acids of the linolenic acid series and the linoleic acid series was thus much lower in the formula fed than in the breast fed infants, the ratio being closely correlated with the dietary linolenate/linoleate ratio. Although the concentration of essential fatty acids was low in both plasma and red cell phosphoglycerides of breast fed infants, there was no increase of 20:3 ($n-9$).

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two substances chosen for the determination of the glomerular filtration rate (GFR) and effective renal plasma flow (RPF) are prepared according to the dosage mentioned above in separate disposable 1 ml plastic syringes and weighed on a Mettler balance to 0.1 mg. In a similar manner an "aliquot" approximating one fifth of the doses of both substances is prepared and weighed to be used as a standard. Both aliquots are injected into a 500 ml volumetric flask which is then filled to the mark with tap water. The two doses are injected rapidly into the clamped infusion tubing and a chronometer is started while the infusion runs fast for approximately 1 minute in order to flush both substances as quickly as possible into the blood vessels. In older children the infusion is then taken away and the needle closed by its stylet while the NaCl infusion will be kept dropping as slowly as possible in younger children all through the examination in order to maintain the vein open. The 4 syringes of both doses and aliquots are then weighed again and the injected amount is calculated.

At regular short intervals (usually 8, 16, 24, 30, 40, 55, 70 and 88 min after the injection of the clearance substances) a 1.5 ml blood sample is drawn through the indwelling needle without using a tourniquet and after centrifugation 0.5 ml of plasma are pipetted into counting tubes. Three samples of 500 μ l of the diluted standard solution are prepared in the same manner (Carlsberg micropipets). All the plasma samples and the standards are measured in an Auto-Gamma (Packard Instruments International) with a 2 channel spectrometer one window being centered on the 324 kev of the photoelectric peak of ^{51}Cr the second one on the 35 kev of the peak of the ^{125}I . The first channel registers only the counts of ^{51}Cr and none of ^{125}I the second window is opened in such a manner that the counts

due to ^{51}Cr registered represent less than 10% of the counts counted in the first channel. This ratio is recontrolled each time the channels are set for counting by determining the activity of a ^{51}Cr standard in both channels. All the samples are then counted for a period of 10 min which gives a precision of over 1% for the standard and the first plasma samples and at least 2.5% for the last ones. After correction of the counts of the second channel for the ^{51}Cr activity the values of each isotope expressed in cpm/ml are plotted on semilogarithmic paper separately for ^{51}Cr EDTA and ^{125}I OH.

Calculation of the clearance

The graphic resolution of the experimental curve is performed as follows (Fig 1): a straight line is fitted through the three or four last points of the plasma disappearance curve. Its intercept with the y axis is determined as well as its half life time defined as the time which passes until the radioactivity has diminished to half its original value. The intercept is called A the half life time $T_{1/2A}$. The first points of the original plasma disappearance curve lie higher than the straight line the value of which is subtracted point by point from the experimentally obtained curve. Thus a second straight line is obtained and its intercept B with the y axis and its half life time $T_{1/2B}$ are also read on the graphic. The value of the clearance is then calculated using following formula:

$$C = \frac{0.693 I}{A T_{1/2A} + B T_{1/2B}} \quad \text{where } I = D \frac{Wd}{Wu} Q$$

I = injected activity in cpm Wd = weight of the injected activity in g Wu = weight of the undiluted aliquot in g Q = activity of the diluted aliquot in cpm/ml D = dilution factor of the aliquot in ml

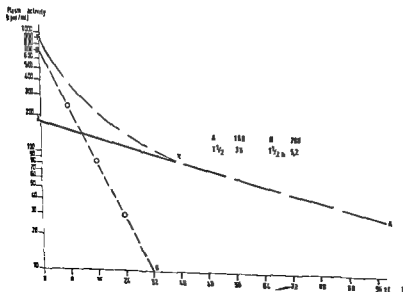


Fig 1 Resolution of the plasma disappearance curve

THE SIMULTANEOUS DETERMINATION IN CHILDREN OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW BY THE SINGLE INJECTION CLEARANCE TECHNIQUE

A DONATH

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Very shortly after Homer Smith (1) had introduced the concept of clearances into medicine, and even before its significance in physiology and hospital routine had been completely recognised, it became evident that the method was often too complicated and the procedure too cumbersome for the patient in order to enjoy a broad acceptance by the physicians. Today the general opinion is unchanged and the complexity of the method still makes a large propagation impractical.

This is one reason why many attempts have been made to simplify the standard technique, either by renouncing to the catheterisation of the bladder or by suppressing the intravenous infusion.

In recent years, surprisingly good results have been reported using a single injection clearance technique (2-17). After 2 years of practice and over 500 clearances performed this way in adults and even more in children, it appears reasonable to us to make a review of our experience as well as of the theoretical basis of this method.

METHODOLOGY

Indicator substances

The commercially available Hippuran or orthoiodohippuric acid labelled with ^{125}I (OIH ^{125}I) is used for

the determination of effective renal plasma flow. Its specific activity is 2 to 6 mCi/mmol of OIH ^{125}I . It has been checked chromatographically for free iodine and even after 4 weeks of cold storage shows less than 2% free ^{125}I . As a glomerular marker ethylenediaminetetraacetate ^{51}Cr (^{51}Cr EDTA) is used dissolved in NaCl containing Na-EDTA 0.0025 molar and with a specific activity of 40-50 mCi/mg Cr. After 1 month of storage there is no free ^{51}Cr detectable. Hippuran has been shown to behave as the classical paraaminohippuric acid (1) and ^{51}Cr EDTA as inulin (12, 18, 19). The following doses of the labelled compounds are used: 40 μCi ^{125}I /m² and 100 μCi ^{51}Cr /m² (this higher dosage being due primarily to the fact that ^{51}Cr has only 12 gamma rays). The photoelectric peak of ^{125}I lies at 35 keV, the one of ^{51}Cr at 324 keV. This difference in the energy of the gamma rays allows an easy spectrometric separation of both isotopes when counted simultaneously. The physical half-life time of both isotopes is 60 and 27 days respectively and this allows to store them and have them available for clearance purposes at any time. However, no solution older than 4 weeks has ever been used.

Single injection procedure

The technique described in adults elsewhere (16) has been taken over in pediatrics with very minimal changes. The clearance determination is performed on hospitalized children as well as on outpatients who just stay for 2 hours in the hospital. A special room of the metabolic ward is devoted to these examinations which are always performed by a specialised medical and nursing team. A saline infusion is started through an indwelling needle placed in a cubital vein and 15 ml of blood is taken which will be used as a blank in order to make sure that there is no radioactivity in the patient's blood. The

stance studied each unit of time will be in case of a mamillary system (2)

$$Cl = \frac{\text{Dose } k_1 k_2}{Ak_2 + Bk_1} \quad (A \text{ and } B \text{ being the respective intercept } (C_1 - C_2) \text{ and } C_2)$$

or by replacing the slopes by the half life time

$$Cl = \frac{\text{Dose } 0.693}{A T_{1/2a} + B T_{1/2b}}$$

In case of a catenary system where the substance must go from compartment 1 into compartment 2 before being excreted the calculation of the excretory flux is only possible if access to compartment 2 is possible as well

What is the correct model for clearances performed by the single injection technique?

Let us examine the experimental reason first. Over 1 200 single injection clearances have been performed in our laboratory during the last 3 years and practically in every case it has been possible to resolve the plasma disappearance curve in two exponentials. This is true for high normal clearance values (the highest GFR was 172 ml/min/1.73 m²) as for very pathological ones the lowest being 7 ml/min/1.73 m². In our experience as well as in that of the other authors a 2-compartment open model cannot be disproved. On the other hand the correlations obtained between clearances performed by single injection technique and by classical ones (3 5 8 9 10 12 14 16 17 18 23) indicate also that the chosen model corresponds to reality.

Another proof for the mamillary system is given by our knowledge of renal physiology. Even if the plasma disappearance curve does

not allow to find out what are the anatomical compartments concerned renal physiology teaches us that substances which are filtered pass directly from the blood through the glomerulum and that the second compartment certainly cannot be located between the blood vessels and the glomerular barrier. The same thought can be applied for the extraction of PAH or OIH from the blood by the renal tubule.

These thoughts are indirect proofs or at least very strong indications that the irreversible excretion occurs from the first compartment and they most likely exclude a catenary system in which the substance studied passes from compartment 1 into compartment 2 before getting excreted. This model therefore appears exact if there are really only 2 compartments.

An objection often made to the single injection method is that if a patient has edema the extracellular fluid may not be only one single compartment and the system will no longer be a bi-compartmental one. In fact one would obtain a plasma disappearance curve composed by 3 exponentials. Even if the graphic decomposition used for the determination of the individual exponentials is too rough to allow a precise separation of this third exponential the way of calculating the excretory flux is still practically correct if the model is of the mamillary type (Fig 3). In fact the value of the denominator is proportional to the surface under the disappearance curve.

Assuming that this possible third compartment would only equilibrate after the 85th minute which represents the latest time the

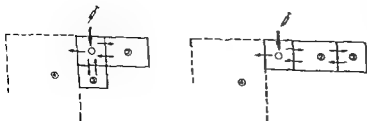


Fig 3 Open 3-compartments model

Theoretical basis of the single injection clearance

Already before the availability of radioactive substances, kinetic studies have been performed in animals and in humans but with substances which are normally not present within the organism as bromsulphalein for the study of the liver (20). If a substance is only sparsely present, an external large load will allow to proceed to the determination of its volume of distribution and its clearance from the blood, and this is what Sapirstein (2) did when he injected creatinine intravenously into dogs.

Tracer techniques or kinetic studies have become a very enriching and broadly spread method for clinical investigations since radio-labelled substances are available. Theoretical models have been experienced in practice and a new approach is now possible, bringing up new concepts, as the measurement of calcium accretion into bone (21) or allowing a simplification of already used techniques, a simplification for the medical staff for the laboratory, and last but not least for the patient. This is the reason for the enthusiastic acceptance of these tracer techniques, but also for misinterpretation and overinterpretation which led to false conclusions and often made some physicians condemn the whole concept of kinetic studies. The single injection technique is based on Sapirstein's experiment (2), who measured the volumes of distribution of intravenously injected creatinine. Sapirstein is very cautious in his paper and explains all the assumptions he makes. Effectively there was no way to control whether they are right or not.

Sapirstein proposes a 2 compartment model. Today one would speak of an opened biological system of 2 compartments placed in parallel also called of a mamillary type. This means that the first compartment into which the labelled substance is introduced is open and not the second one. Only under such condition the mathematical treatment of Sapirstein's model is correct. If the opening is in the second compartment one calls this a catenary model or a 2 compartment system in series (Fig. 2). Actually the system opens into a third compartment, so huge that the labelled substance will never reach a detectable equilibrium. In the mamillary model the labelled substance injected into the first compartment can either go into compartment 2 or into compartment 3. In the catenary model it must pass through compartment 2 in order to reach compartment 3. In both cases what gets into 3 will not get back, this passage is irreversible for this reason the model is called an open 2 compartment system.

The mathematical treatment of a mamillary and of a catenary open 2 compartment model is similar up to a certain point but differs in some others. The plasma disappearance curve is in both cases the sum of two exponentials, the concentration in the plasma at any time t is given by the formula:

$$C_t = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda t}$$

where $(C_1 - C)$ and C_2 are the logarithms of the intercepts with the y axis and λ_1 and λ the slopes of the rapid and slow exponentials respectively.

The clearance (Cl), which corresponds to the amount of fluid cleared from the sub-

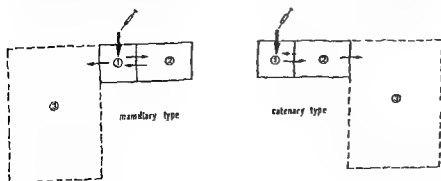


Fig. 2 Open 2 compartments model

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In case of a catenary system where the substance must go from compartment 1 into compartment 2 before being excreted the calculation of the excretory flux is only possible if access to compartment 2 is possible as well

What is the correct model for clearances performed by the single injection technique?

Let us examine the experimental reason first. Over 1 200 single injection clearances have been performed in our laboratory during the last 3 years and practically in every case it has been possible to resolve the plasma disappearance curve in two exponentials. This is true for high normal clearance values (the highest GFR was 172 ml/min/1.73 m) as for very pathological ones the lowest being 7 ml/min/1.73 m. In our experience as well as in that of the other authors a 2-compartment open model cannot be disproved. On the other hand the correlations obtained between clearances performed by single injection technique and by classical ones (3 5 8 9 10 12 14 16 17 18 23) indicate also that the chosen model corresponds to reality.

Another proof for the mamillary system is given by our knowledge of renal physiology. Even if the plasma disappearance curve does

not allow to find out what are the anatomical compartments concerned renal physiology teaches us that substances which are filtered pass directly from the blood through the glomerulum and that the second compartment certainly cannot be located between the blood vessels and the glomerular barrier. The same thought can be applied for the extraction of PAH or OIH from the blood by the renal tubule.

These thoughts are indirect proofs or at least very strong indications that the irreversible excretion occurs from the first compartment and they most likely exclude a catenary system in which the substance studied passes from compartment 1 into compartment 2 before getting excreted. This model therefore appears exact if there are really only 2 compartments.

An objection often made to the single injection method is that if a patient has edema the extracellular fluid may not be only one single compartment and the system will no longer be a bicompartimental one. In fact one would obtain a plasma disappearance curve composed by 3 exponentials. Even if the graphic decomposition used for the determination of the individual exponentials is too rough to allow a precise separation of this third exponential the way of calculating the excretory flux is still practically correct if the model is of the mamillary type (Fig 3). In fact the value of the denominator is proportional to the surface under the disappearance curve.

Assuming that this possible third compartment would only equilibrate after the 85th minute which represents the latest time the

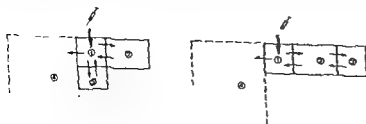


Fig 3 Open 3-compartment model

Theoretical basis of the single injection clearance

Already before the availability of radioactive substances, kinetic studies have been performed in animals and in humans but with substances which are normally not present within the organism as bromsulphalein for the study of the liver (20). If a substance is only sparsely present, an external large load will allow to proceed to the determination of its volume of distribution and its clearance from the blood and this is what Sapirstein (2) did when he injected creatinine intravenously into dogs.

Tracer techniques or kinetic studies have become a very enriching and broadly spread method for clinical investigations since radio-labelled substances are available. Theoretical models have been experienced in practice and a new approach is now possible, bringing up new concepts as the measurement of calcium accretion into bone (21) or allowing a simplification of already used techniques, a simplification for the medical staff for the laboratory, and last but not least for the patient. This is the reason for the enthusiastic acceptance of these tracer techniques, but also for misinterpretation and overinterpretation which led to false conclusions and often made some physicians condemn the whole concept of kinetic studies. The single injection technique is based on Sapirstein's experiment (2), who measured the volumes of distribution of intravenously injected creatinine. Sapirstein is very cautious in his paper and explains all the assumptions he makes. Effectively there was no way to control whether they are right or not.

Sapirstein proposes a 2-compartment model. Today, one would speak of an opened biological system of 2 compartments placed in parallel, also called of a mamillary type. This means that the first compartment into which the labelled substance is introduced is open and not the second one. Only under such condition the mathematical treatment of Sapirstein's model is correct. If the opening is in the second compartment, one calls this a catenary model or a 2 compartment system in series (Fig. 2). Actually the system opens into a third compartment, so huge that the labelled substance will never reach a detectable equilibrium. In the mamillary model the labelled substance injected into the first compartment can either go into compartment 2 or into compartment 3. In the catenary model it must pass through compartment 2 in order to reach compartment 3. In both cases what gets into 3 will not get back; this passage is irreversible. For this reason the model is called an open 2 compartment system.

The mathematical treatment of a mamillary and of a catenary open 2 compartment model is similar up to a certain point but differs in some others. The plasma disappearance curve is in both cases the sum of two exponentials; the concentration in the plasma at any time t is given by the formula:

$$C_t = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}$$

where $(C_1 - C_2)$ and C are the logarithms of the intercepts with the y-axis and k_1 and k_2 the slopes of the rapid and slow exponentials respectively.

The clearance (Cl), which corresponds to the amount of fluid cleared from the sub-

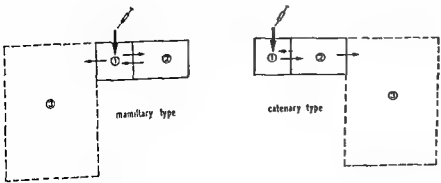


Fig. 2 Open 2 compartments model

Table 1 Single injection clearance *Reproducibility*

C. D. 14 years			H. G. 11 years			A. P. 9 years		
(ml/min/1.73 m ²)			(ml/min/1.73 m ²)			(ml/min/1.73 m ²)		
Date	GFR	RPF	Date	GFR	RPF	Date	GFR	RPF
31.3	110	584	4.2	107	622	11.2	124	592
2.4	104	590	5.2	98	594	12.2	131	599
4.4	104	594	6.2	104	603	13.2	115	592
8.4	95	568	7.2	111	624	14.2	117	603
11.4	101	583						
Mean value	102.8	584.8		105.0	610.75		121.75	596.5
S.D.	5.45	10.0		5.48	13.93		7.28	5.45
S.D. (%)	5.30	1.71		5.22	2.26		5.96	0.92

proofs have been brought up in humans not even in normal individuals

In practice however even if the 2-compartment open mamillary model used for calculation does not correspond to reality in all cases the resulting error will be so small that it lies within the limitation of any technique as complicated as the determination of the renal clearances including the classical way of determination

Reproducibility

In one child the renal clearances are determined by single injection technique 5 times within 12 days (Table 1). The results show a standard deviation of 5.3 for the glomerular filtration rate and 1.7 for the RPF. In two other children the test is performed once a day 4 days in a row and shows for GFR a standard deviation of 5.5 and 5.7% respectively and for RPF 2.3 and 0.9. With a 95% confidence the GFR result varies between 10.6 and 11.4 and the RPF between 1.8 and 4.6%. If one remembers that there are no basic conditions for clearance determinations it may be useless to determine the reproducibility of clearances in one single person (27) but our results still fall within the limits of what others have found (14).

Simplified techniques

Many authors (3, 8, 10, 13, 28) despising the mathematical bases of the tracer and com-

partment models have oversimplified the single injection technique but as already shown in a previous paper (6) such an oversimplification can only occur on the account of accuracy. In Fig. 5 we have represented a case in which the ⁵¹Cr-EDTA equilibrates very rapidly within the second compartment so that after 24 min both spaces are in equilibrium. The clearance value is indirectly proportional to the surface under the disappearance curve marked by *YA* (this last point lies theoretically at the infinite on the straight line *AA'*) with the simplified method which measures the plasma concentration after 8 and 18 min only the triangle *BB'O* is delimited which obviously is much smaller than the correct surface and therefore will give a too elevated clearance value. Both the 40/60 min method and the 20/30 min method will end up with a result which will be slightly too high as the surface delimited this way underestimates the effective one. On Fig. 5 we have a case in which the equilibrium between compartments 1 and 2 is only obtained after 54 min. The effective surface is delimited by *YAO*. Again the 8/18 min method delimits the triangle *BB'O* and the 20/30 min the triangle *CC'O* both of them being by far too small and giving an overestimated renal clearance. The 40/60 method on the other hand delimits the triangle *DD'O* which corresponds more or less to the triangle *AA'O* and only neglects the part of the surface corresponding to *YOA*. From these two examples it is obvious that

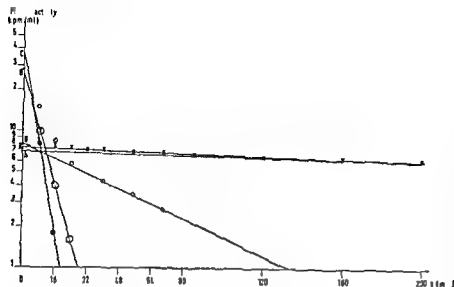


Fig 4 Plasma disappearance curve of a 3 compartments model in which a third exponential appears after the 85th minute. The error by missing it is represented by the black surface between the two lines after the 180th minute. In this case it would only make a difference of 3 ml/min on the final clearance result.

plasma activity is determined with our technique, it would only cause a minimal modification of the final results of the clearance and this slight change would be a diminution of the clearance value, as the appearance of the third exponential would slightly increase the surface under the disappearance curve (Fig 4).

Again the model is mathematically not absolutely correct if we have a combined mamillary-catenary 3 compartment system (Fig 3) where the excretion happens from the first compartment, but the third one is not directly related to the first one, but to the second one. In such a case, the model proposed by Sapirstein is once more mathematically not absolutely exact, but the error in missing the third exponential would be so small that it would fall within the usual precision of the method. If one assumes that the first compartment is plasma and the others form the extracellular fluid, such a model would appear absolutely compatible with our physiologic knowledge, showing that the extracellular fluid is not a single compartment, as it is no longer homogenous for the substances studied which would first equilibrate within a more rapidly exchangeable compartment and then within a slower exchangeable one, which could well be represented by edema. But it is known that water (24) and substances like thiosulfate (25) or inulin (26), which equilibrate within the extracellular fluid need 4 to 8 hours to ex-

change completely, for this reason the single injection technique proposed, in which the plasma disappearance curve is only measured for a duration of 85 minutes, does not allow to reach this complete equilibration and the calculation of the size of the compartments leads to figures which do not correspond to any known anatomical space (2, 16).

All these are empirical indirect proofs of the practical exactitude of the mathematical model. The irrefutable demonstration of what the absolutely exact model is, can only be brought by one of the following two experiments.

(a) The theory of a bicompartimental open model teaches us that the specific activity in compartment 2 will start at 0, go up to reach maximum and then decrease again. If this specific activity determined once during the decreasing period is higher than in the first compartment at the same time, the system is of the mamillary type. If it is lower it is a catenary model.

(b) When the specific activity in the first compartment and in the excreted fluid (primary urine in the case of renal clearances) are determined at the same time, the specific activity of the excreted material is lower than the one inside compartment 1 when the system is a catenary one; they are equal if the mamillary system is correct.

To our knowledge so far none of these two

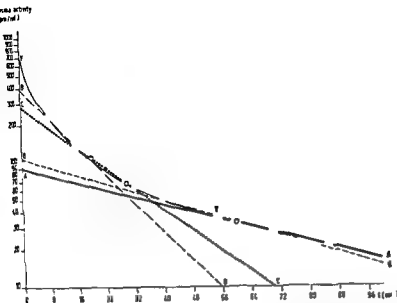


Fig 6 Bi-exponential method compared to simplified techniques II

of the indications of the determinations of glomerular filtration rate and effective renal plasma flow. A more frequent determination of the kidney function in progredient renal diseases or during recovery from an acute illness or before and after a nephrectomy will help the pediatrician to follow his renal patients better. The discomfort of the method is not much greater than a simple venous puncture so that his patient and especially the boys will no longer feel reluctant to iterative clearance determinations. On the other hand there are very specific indications for this single injection method which are detailed in a further paper (23).

SUMMARY

The performance of over 1 000 clearances with the single injection method allows a retrospective review on the value of this new technique. The choice of the indicator substance, the reproducibility of the method and the irradiation dosimetry do not bring up very exciting problems. The theoretical basis of the justification of the single injection method has not been proven so far and the inexactitude which may happen because of the empirical assumption

that the substances used behave as in an open mamillary bicompartamental model is discussed. The author comes to the conclusion that although the rightness of the model has never been proven the method will give sufficient precise results in practice and is proposed to a large extension because of its technical simplicity as well as for the patient as for the physician and the laboratory technician. The irradiation dose is so small that it will practically never be considered as a contra indication.

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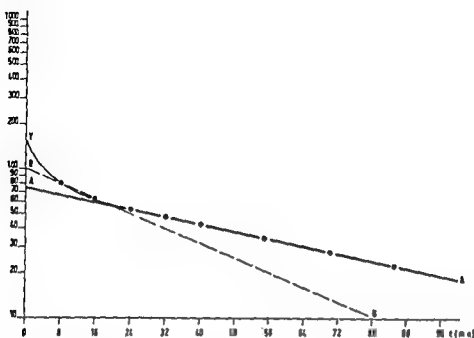
Plasma activity
(cpm/ml)

Fig 5 Bi exponential method compared to simplified techniques 1

these simplified methods can be right one time or another, but that they are subject to great errors depending of the rapidity of the equilibration of compartment 1 and compartment 2. On purpose we did not mention whether these curves are obtained with ^{51}Cr EDTA or $\text{OIH-}^{131}\text{I}$ because the same way of thinking will apply in both cases.

Radiation dosimetry

The absorbed dose of both radioactive substances has been calculated according to the latest knowledge in the field of dosimetry (29), using Lowinger's (30) method of calculation. We have postulated that the urine is evacuated from the bladder two hours after getting there; this assumption is based on frequent controls performed during routine procedures. With a normal renal function the single injection clearance method causes a total body irradiation of less than 10 mrad and in cases with a very high impairment ($\text{GFR} = 10 \text{ ml/min/1.73 m}^2$) an irradiation of less than 60 mrad. These figures are valid for all ages as the dose of the radioisotope is proportional to the body surface. This irradiation lies under the dosimetry of a thoracic radiography: a fortunate person with a normal kidney function, who

spends 3 weeks skiing in Adelboden (alt 1500 m) rather than working in Berne (alt 500 m) will get more irradiated by cosmic rays than by the isotopes of a single injection clearance.

Indications

The single injection method is particularly welcome in the pediatric field, as especially in boys the catheterisation of the bladder always represents a psychologically dramatic procedure. There is no doubt that for the patients it represents a much less uncomfortable examination than the classical clearances. So far the routine procedure has been in pediatric clinics to limit the inulin and PAH clearances to selected cases and to use the endogenous creatinine clearance as a rough screening test for glomerular filtration rate. Between the exactitude of the standard inulin and PAH clearances, which is by far not absolute and the great inaccuracy of the creatinine clearance there is a large no man's land in which the single injection clearance in our opinion appears to stay very close to the classical clearance methods. Because of its technical simplicity, this single injection technique should become more and more popular and will certainly allow to extend the field

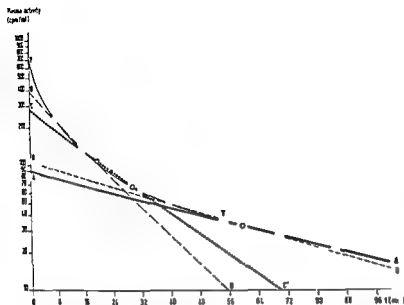


Fig 6 Bi-exponential method compared to simplified techniques II

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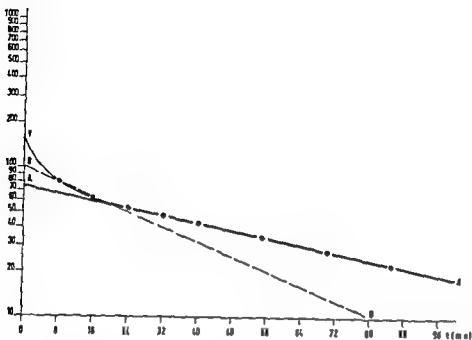
Plasma activity
(cpm/ml)

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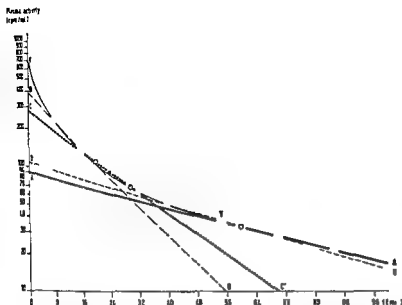


Fig 5 Bi-exponential method compared to simplified techniques II

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Key words Glomerular filtration rate effective renal plasma flow clearance technique

SUBCLINICAL DEFECTS IN RENAL REGULATION OF ACID BASE BALANCE IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS

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One principal function of the kidney is its contribution to the maintenance of the acid base balance (9, 10). This is accomplished by the secretion of hydrogen ions which either participate in the reabsorption of bicarbonate or are excreted in the form of ammonium ions or titratable acids.

Renal insufficiency is almost always accompanied by acidosis (10, 11). In patients with moderately severe renal disease where renal function is sufficient to protect from azotemia, clinical manifest acidosis is however generally not observed. It is likely though that the renal acidosis in uremia is the final result of a progressive lesion of the urinary acidifying mechanisms. A reduced protection against non renal acidosis can therefore be expected in patients with moderate reduction of renal function. To test this short time response to ammonium chloride induced metabolic acidosis has been studied in a group of children (age range 5 to 17 years) with recurrent urinary tract infections and with glomerular filtration rates (GFRs) ranging between 28 and 132 ml/min/1.73 m² body surface (b.s.). For reference a group of normal children has also been studied

(age range 6 to 16 years) had previous histories of urinary tract infections confirmed by urine cultures. They were however without any clinical or bacteriological signs of urinary tract infection the last 2 months preceding the study. In none of the patients any gross abnormality in calcium and phosphorus homeostasis was found. Fifteen of these children with histories of recurrent urinary tract infections had been submitted to previous clearance studies that have been reported in detail elsewhere (1, 2, 3). In these 15 patients the glomerular filtration rate (GFR) during water diuresis had been determined by the clearance of inulin. In the remaining patient the GFR had been determined by the 24 hour clearance of endogenous creatinine. In addition 11 children (age range 6 to 16 years) with no histories of renal disease or other disorders affecting the electrolyte or acid base balance of the body were also studied. The results from this group of children were used as normal values. Those children were all within normal limits for height and weight. Informed consent to the studies were obtained from all the patients and their parents.

METHODS

All the children were kept on a constant salt intake 3 days prior to the study. A modification of the short ammonium chloride test described by Edelmann et al. was used (4). The study was started after a standard breakfast meal. In order to keep a constant urine flow rate the patients were given water in an amount of 0.1-0.2 ml/min/kg b.w. The forced fluid intake was started 2-4 hours before the intake of ammonium chloride. Urine was collected hourly by spontaneous voiding. Following control sampling of blood and urine ammonium chloride was given orally in the amount of 150 mEq/m² b.s. After the administration of the ammonium chloride another 5 urine samples were collected and 2-3 blood samples were taken 2, 3 and 5 hours after the administration. Immediately after voiding the urine samples were with

MATERIAL

Twenty seven children, 11 boys and 16 girls, aged 5 to 17 years were studied. Sixteen of the children

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SUBCLINICAL DEFECTS IN RENAL REGULATION OF ACID BASE BALANCE IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS

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One principal function of the kidney is its contribution to the maintenance of the acid base balance (9 10) This is accomplished by the secretion of hydrogen ions which either participate in the reabsorption of bicarbonate or are excreted in the form of ammonium ions or titratable acids

Renal insufficiency is almost always accompanied by acidosis (10 11) In patients with moderately severe renal disease where renal function is sufficient to protect from azotemia clinical manifest acidosis is however generally not observed It is likely though that the renal acidosis or uremia is the final result of a progressive lesion of the urinary acidifying mechanisms A reduced protection against non renal acidosis can therefore be expected in patients with moderate reduction of renal function To test this short time response to ammonium chloride induced metabolic acidosis has been studied in a group of children (age range 5 to 17 years) with recurrent urinary tract infections and with glomerular filtration rates (GFRs) ranging between 28 and 132 ml/min/1.73 m body surface (bs) For reference a group of normal children has also been studied

MATERIAL

Twenty-seven children 11 boys and 16 girls aged 5 to 17 years were studied Sixteen of the children

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(age range 6 to 16 years) had previous histories of urinary tract infections confirmed by urine cultures They were however without any clinical or bacteriological signs of urinary tract infection the last 2 months preceding the study In none of the patients any gross abnormality in calcium and phosphorus homeostasis was found Fifteen of these children with histories of recurrent urinary tract infections had been submitted to previous clearance studies that have been reported in detail elsewhere (1 2 3) In these 15 patients the glomerular filtration rate (GFR) during water diuresis had been determined by the clearance of inulin In the remaining patient the GFR had been determined by the 24 hour clearance of endogenous creatinine In addition 11 children (age range 6 to 16 years) with no histories of renal disease or other disorders affecting the electrolyte or acid base balance of the body were also studied The results from this group of children were used as normal values Those children were all within normal limits for height and weight Informed consent to the studies were obtained from all the patients and their parents

METHODS

All the children were kept on a constant salt intake 3 days prior to the study A modification of the short ammonium chloride test described by Edelmann et al was used (4) The study was started after a standard breakfast meal In order to keep a constant urine flow rate the patients were given water in an amount of 0.1-0.2 ml/min/kg bw The forced fluid intake was started 2 to 4 hours before the intake of ammonium chloride Urine was collected hourly by spontaneous voiding Following control sampling of blood and urine ammonium chloride was given orally in the amount of 150 mEq m bs After the administration of the ammonium chloride another 5 urine samples were collected and 2-3 blood samples were taken 2 3 and 5 hours after the administration Immediately after voiding the urine samples were with

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Table 2 Details of a study on the effect of an oral intake of ammonium chloride in a normal child

Time (min)	Urine pH	Blood pH	Standard bicarbonate (mM/l)	P _{CO} (mm Hg)	Total CO ₂ (mM/l)	Ammonia excretion (μ Eq/mm/1.73 m ² s)
-120	Start of urine sampling					
-60	7.02	7.41	25.0	41	27.0	5
0	6.95	7.39	24.0	41	26.0	
	150 mEq/m ² s ammonium chloride given by mouth					
60	6.37					
120	5.93	7.36	23.0	41	24.0	29
180	5.15	7.35	21.0	38	21.8	
240	4.85	7.30	18.0	35	17.9	54
300	4.82	7.29	17.0	35	17.4	54

from a typical study in a normal child is given in Table 2. The control total CO₂ concentration i.e. before the ammonium chloride was given ranged between 23 and 30 mM/l. The development of acidosis is manifested by depression of total CO₂ concentration had generally started 3 hours after the administration of ammonium chloride. Five hours after the administration of ammonium chloride the total CO₂ concentration ranged between 15 and 20 mM/l in all but one child. The relationship between total CO₂ concentration and urine pH in normal children was characteristic. A sharp fall in urine pH was noticed when the total CO₂ was depressed from 25 to 21 mM/l. When total CO₂ concentration fell beyond 18 mM/l generally no further fall in the urine pH was noted.

The results from the patients with recurrent urinary tract infections and low to moderately reduced filtration rates are demonstrated in

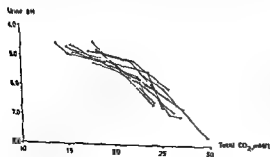


Fig. 1 The relationship between blood total CO₂ and urine pH in 11 normal children.

Fig. 2 and Table 3. Table 3 records a typical experiment in a patient with low GFR (1 L/L). Fig. 2 demonstrates the relationship between the total CO₂ concentration and urine pH in the patients with low GFRs. The total CO₂ concentration before the ammonium chloride was given ranged between 19.5 and 25.5 mM/l. In 4 of the 5 patients the total CO₂ concentration had to be depressed well below 20 mM/l before the urine hydrogen ion secretion increased i.e. the urine pH was lowered. In one of these patients (S.N.) the total CO₂ concentration had to be depressed as low as 11 mM/l before a fall in urine pH was achieved. The remaining patient did not demonstrate a fall in urine pH despite the depression of total CO₂ concentration to 12.5 mM/l. Thus 4 of 5 patients with low filtration rates demonstrated principally the same relationship between total CO₂ concentration and urine pH as the normal controls but their curve was displaced to the left i.e. the urine pH was depressed at a much lower total CO₂ concentration.

The results from the patients with recurrent urinary tract infections and subnormal to normal GFRs are summarized in Fig. 3 which demonstrates the relationship between total CO₂ concentration and urine pH. Six of the patients showed the same relationship between the total CO₂ concentration and urine pH as the normal children. A further 4 of the 11 patients were also able to acidify the urine but

Table 1 Glomerular filtration rate and basal acid base data in all patients studied

Patients	Age (years)	Sex	Clinical grouping ^a	GFR (ml/min/1.73 m ²)	Serum calcium (mEq/l)	Blood pH	P _{co} (mm Hg)	Standard bicarbonate (mM/l)
S N	11 1/12	M	B	28	4.3	7.25	44	18
K H	16 8/12	F	C	32	4.8	7.40	31	21
I L L	12 1/12	F	B	39	4.4	7.33	42	21
U K	13 0/12	M	B	50	4.8	7.40	40	25
L N	11 4/12	F	C	51	4.7	7.40	37	23
I B S	9 10/12	F	B	82	4.7	7.39	44	26
A K F	10 5/12	F	B	92	4.7	7.40	39	24
B S	9 0/12	F	B	96	5.1	7.40	39	24
E J	11 11/12	F	B	98	4.7	7.38	45	25
K O	7 0/12	F	C	104	4.8	7.42	34	23
I O	11 10/12	F	B	110	4.6	7.40	36	23
A E	14 4/12	F	C	112	4.5	7.37	41	23
M I	8 6/12	F	B	115	—	7.32	37	19
S A	9 4/12	F	B	122	—	7.37	40	23
M K	6 8/12	F	C	131	—	7.38	39	23
A K B	7 6/12	F	B	132	4.4	7.41	39	25

^a Group A Clinical history of at most one infection with or without significant bacteriuria. Group B Clinical history of one to three urinary tract infections yearly. Group C Clinical history of three or more infections yearly confirmed by urine cultures or recurrent episodes of high fever and one sided abdominal pain radiating dorsally and simultaneous bacteriuria.

drawn and kept anaerobically on ice pH analysis were carried out within 15 min of voiding. The pH was determined on a pH meter 26 (Radiometer). Pre-warmed capillary blood samples were taken and analysed for actual pH and pH in samples equilibrated with 4% and 8% CO₂ on a pH meter 27 (Radiometer). By plotting these data on a Siggaard Andersen curve nomogram P_{co}, standard bicarbonate concentration and blood total CO₂ concentration could be obtained (15). In 6 normal children and 14 of the patients each urine sample was analysed for ammonia by the colorimetric phenate hypochlorite method (7).

RESULTS

Table 1 demonstrates blood pH, standard bicarbonate concentration and P_{co} from all the patients in control blood samples before ammonium chloride was given. The glomerular filtration rate per 1.73 m² body surface during water diuresis and a clinical summary are also included. The clinical classification is that used in a previous report (3). It is evident that the patients cover a wide range of filtration rates and have rather heterogeneous clinical histories. There is no good relationship between the frequency of urinary tract infections and the degree of reduction of GFR.

Three of the children with recurrent urinary tract infections (S N, I L L, M I) revealed laboratory signs of mild uncompensated metabolic acidosis. One of the children (K H) had laboratory signs of compensated metabolic acidosis. In the remaining children the pH, P_{co} and standard bicarbonate concentration were within normal limits.

The results of the short ammonium chloride test are best interpreted by relating the urine pH to the blood total CO₂ concentration (4). When evaluating this relationship we have compared the results from the normal children with those from two different groups of children with recurrent urinary tract infections, namely (1) five children with low to moderately reduced (low) filtration rates (S N, K H, I L L, L N and U K) and (2) eleven children with subnormal to normal (high) filtration rates (I B S, A K F, B S, E J, K O, I O, A E, M I, S A, M K and A K B).

The results from the normal children are demonstrated in Fig. 1 and Table 2. Fig. 1 shows the normal relationship between total CO₂ concentration and urine pH. A protocol

Table 4 The maximal ammonia excretion observed. The values obtained during either of the two last urine collection periods

	Ammonia excretion ($\mu\text{Eq}/\text{min}/1.73 \text{ m}^2$)	Ammonia excretion ($\mu\text{Eq}/100 \text{ ml}$ glomerular filtrate)
Normal children	51 ± 12	63 ± 26^a
Patients with low GFRs	23 ± 6	44 ± 17^a
Patients with high GFRs	45 ± 12	
Normal children vs patients with low GFRs	$p < 0.01$	$p > 0.3$
Normal children vs patients with high GFRs	$p > 0.3$	$p > 0.4$
Patients with low GFRs vs patients with high GFRs	$p < 0.01$	$p > 0.1$

^a Glomerular filtration rate not measured

^b Glomerular filtration measured at previous clearance studies

fore the ammonium chloride was given. All the normal children and all the patients with high filtration rates were able to respond to a depression in urinary pH with an increase in ammonia excretion. All the patients with low GFRs were able to increase the ammonia excretion as the urine pH was lowered. In 2 of the 5 patients, however, the increase was much lower than what was observed in any of the normal children and in any of the patients with high GFRs.

DISCUSSION

Generally three defects of the renal acidifying mechanisms have been recognized: (1) Reduced ability to reabsorb filtered bicarbonate. This defect has been reported both in patients

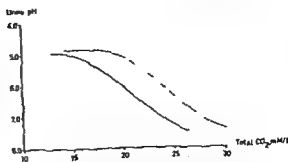


Fig. 5 The theoretical relationship between blood total CO_2 and urine pH in a normal control (—) and a patient with reduced bicarbonate threshold (---).

with generalized renal failure (16, 18) and in patients with primary tubular acidosis of so-called proximal type (14). (2) An inability to establish a sufficient hydrogen ion concentration gradient between blood and tubular fluid in the distal tubule. This defect has been reported mainly in patients with primary renal tubular acidosis of so-called distal type (12, 6). (3) An inability of sufficient ammonia excretion. This defect has mainly been reported in patients with generalized renal failure (17, 10). Patients with a defect type (1) but intact "distal hydrogen ion secretion" will be able to lower their urine pH once the renal threshold is passed. Thus the relationship between blood bicarbonate or total CO_2 concentration and urine pH in children with a lowered threshold value for bicarbonate is principally the same as in normal children but the curve is displaced to the left (Fig. 5). From this figure the renal bicarbonate threshold value can be roughly estimated as the total CO_2 concentration where the urine pH starts to fall steeply. Patients with a defect in distal hydrogen ion secretion are unable to increase the hydrogen ion concentration in urine i.e. depress the urine pH (Fig. 6). Patients with defect type (3) present a reduced ammonia excretion but are generally able to acidify their urine because of a compensatory increase in excretion of titratable acids (10). All the recognized dysfunctions in the renal regulation of acid-base balance should thus be discovered by the combined determination of the maximal ammonia

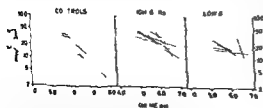


Fig. 4 The relationship between urine pH and the basal and maximal ammonia excretion in 6 normal children: (a) 7 patients with subnormal to normal GFRs; (b) and 5 patients with low to moderately reduced GFRs; (c). The scale is in $\ln \log$.

Table 3 Details of a study on the effect of an oral intake of ammonium chloride in a patient with low GFR

Time (min)	Urine pH	Blood pH	Standard bicarbonate (mM/l)	P _{CO} (mm Hg)	Total CO ₂ (mM/l)	Ammonia (μ Eq/min/1.73 m ² b s)
-120	Start of urine sampling					
-60	6.12					
0	6.01	7.33	21.0	42	23.0	17
	150 mEq/m ² b s ammonium chloride given by mouth					
60	6.01					
120	5.99					17
180	5.95	7.32	17.9	33	17.5	16
240	5.45					
300	5.19	7.26	15.5	33	15.3	19

at lower total CO₂ concentrations than that found in the normal controls. One of the 11 patients reached a total CO₂ concentration of 14.2 mM/l towards the end of the test. At this total CO₂ concentration the urine pH was 6.0.

The maximal ammonia excretion has been evaluated statistically in Table 4. The individuals have been grouped as before, i.e. one group of 6 normal children, one group of 5 patients with recurrent urinary tract infections and low GFRs and one group of 7 patients with recurrent urinary tract infections with high GFRs. The maximal ammonia excretion in normal children averaged $51 \pm 12 \mu\text{Eq/min/1.73 m}^2 \text{ b s}$ (mean \pm one S.D.). In the children with recurrent urinary tract infections and low GFRs the maximal ammonia excretion was significantly lower than that of normal controls. If, however, the maximal ammonia excretion

in the patients with low GFRs is calculated per 100 ml glomerular filtrate there is no significant difference from the normal values. The maximal ammonia excretion both in absolute values and calculated per 100 ml glomerular filtrate in patients with recurrent urinary tract infections and high GFRs do not differ significantly from the normal values. In comparing the patients with low and high GFRs it was found that the absolute maximal ammonia excretion was significantly lower in the patients with low GFRs than in the patients with high GFRs. The maximal ammonia excretion related to filtration rate was, however, not significantly different in the patients with low and high filtration rates.

Fig. 4 demonstrates the relationship between the urine pH and the basal and maximal ammonia excretion in the children studied. The basal ammonia excretion is that obtained be

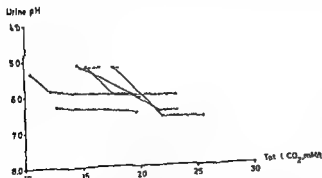


Fig. 2 The relationship between blood total CO₂ and urine pH in 5 patients with low to moderately reduced filtration rates (range 28–51 ml/min/1.73 m² b s). --- outer limits of the values found in normal children.

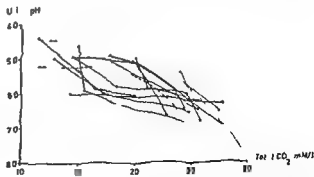


Fig. 3 The relationship between blood total CO₂ and urine pH in 11 patients with subnormal to normal filtration rates (range 82–132 ml/min/1.73 m² b s). --- outer limits of the values found in normal children.

tly reduced filtration rates and four of the patients with subnormal to normal filtration rates demanding larger reduction in blood total CO concentration than the normal controls before a fall in the urine pH could be observed. This finding was taken as a sign of reduced bicarbonate threshold. It is therefore suggested that the reabsorption of filtered bicarbonate is one of the most vulnerable functions of the kidney in recurrent urinary tract infections.

ACKNOWLEDGEMENT

The authors are indebted to Miss Lill-Britt Jonsson, Miss Eivor Sundqvist and Mrs Brita Söderqvist for excellent technical assistance.

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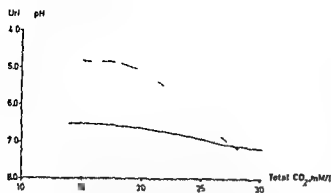


Fig 6 The theoretical relationship between blood total CO_2 and urine pH in normal controls (---) and patients unable to excrete hydrogen ions against a gradient (---)

excretion and of the relationship between blood total CO_2 concentration and urine pH. Analysis of the relationship between total CO_2 concentration and urine pH revealed abnormalities not only in all the patients with low to moderately reduced filtration rates but also in 5 of the patients with subnormal to normal filtration rates. In 4 of 5 patients with low GFRs and in 4 of the patients with high GFRs this abnormality consisted of a displacement of the relationship i.e. the urine was acidified but at a lower total CO_2 value than in the normal children. Those children thus show signs of a reduced bicarbonate threshold. High bicarbonate excretion despite low plasma bicarbonate values has previously been reported in uremic acidosis (16). The results from this study present further evidence that the renal bicarbonate threshold is reduced in renal disease, in this case recurrent urinary tract infections. In fact renal bicarbonate threshold appears to be affected early in the disease process and probably before the filtration rate is reduced. The present study does not allow any further speculations on the nature of the changes in hydrogen ion secretion participating in the reabsorption of filtered bicarbonate in recurrent urinary tract infections. It is however, of interest that a histochemical study of carbonic anhydrase has indicated a reduced enzyme activity in renal disease (8).

Most of the patients both with high and low filtration rates were able to finally de-

press the urine pH to values below 5.5 (10). Thus the ability to establish a high hydrogen ion concentration in urine appeared to be intact even when the filtration rate was reduced. Two patients however, one with low and one with high filtration rate were not at all able to lower urine pH during the course of the study despite the depression of total CO_2 concentration as low as 12–14 mM/l. These 2 patients might thus have a defect in distal hydrogen ion secretion. Very severely reduced bicarbonate thresholds with values below 14 mM/l can however, not be excluded.

Studies on the maximal ammonia excretion revealed a reduction only in patients with low filtration rates. When the ammonia excretion was related to the filtration rate this reduction was however no longer apparent. All the patients with recurrent urinary tract infections that had subnormal to normal filtration rates demonstrated normal maximal ammonia excretion. It has previously been suggested that the reduction in ammonia excretion generally found in uremic acidosis is a quantitative defect secondary to reduced nephron population (10). The ammonia excretion per functioning nephron has thus been assumed to be nearly normal. A selective qualitative defect in ammonia excretion has not been demonstrated. Our finding of a normal ammonia excretion filtration ratio favours the theory of a quantitative reduction of ammonia excretion in renal disease.

SUMMARY

Renal control of acid base balance has been evaluated in children with recurrent urinary tract infections. For reference a group of normal children has also been studied. The studies were performed before and after a single oral intake of ammonium chloride. The following parameters have been determined: the maximal ammonia excretion and the relationship between blood total CO_2 concentration and urine pH. The maximal ammonia excretion was found to be reduced in proportion to the filtration rate. All the patients with low to modera-

Davidson & Sackner (16) PAH was determined according to the method of Bratton & Marshall (16)

Single injection clearance with ^51Cr EDTA and ^{125}I OIH

Cr-ethylenediaminetetraacetate (^51Cr EDTA) was used as indicator substance for GFR and ^{125}I Iodine marked orthoiodohippuric acid (^{125}I OIH) was used as clearance substance for determination of ERPF. The doses for the single injection clearance approximated 100 μCi ^51Cr and 40 μCi ^{125}I . Each of the substance was prepared in a 1 ml disposable syringe. At the beginning of the first period of simultaneous inulin and PAH-clearance they were injected into the tubing of a saline infusion that was connected to the cannulized vein for 2 min in order to insure rapid and complete intravenous application of these substances. The addition of saline did never exceed 10 ml. Timing of the single injection clearances started with the isotope injection. Two ml blood samples were drawn from the indwelling needle placed on the contralateral arm as for the inulin and PAH-clearances. Nine blood samples were obtained first a control before injection then 8, 16, 24, 32, 40, 55, 70 and 85 min after injection of the clearance substances. The radioactivity of the plasma was counted in a well type scintillation counter as described earlier (27).

Statistical evaluation

Depending on the underlying system the model of a functional linear relationship was chosen. There is an exact mathematical relationship between the two obtained clearance values x representing the measurements of the single injection technique and y representing the clearance of inulin or PAH respectively. Thus it was possible to determine whether the results of the single injection technique correspond to those of the inulin- and the PAH-clearance and to calculate the standard error of the regression coefficient. With this model the 95% confidence limits corresponding to the inulin or PAH-clearance can be determined for a single future observed value obtained by single injection clearance. The following formulas are used:

$$\text{Regression coefficient } b_1 = \frac{\bar{y}}{\bar{x}}$$

$$\text{Standard error of regression coefficient } s_{b_1} = \frac{s}{\sum_{i=1}^n x_i^2}$$

$$\text{whereby } (n-1)s^2 = \sum_{i=1}^n (y_i - \frac{\sum_{i=1}^n y_i}{n})^2 - \frac{(\sum_{i=1}^n y_i)^2}{n}$$

The above mentioned 95% confidence limits for a future observed value were calculated according to the following formula:

$$b \pm t \cdot \sqrt{s^2 + (x')^2 s_{b_1}^2}$$

whereas Student's $t_{0.975}$ was taken from tables in the original literature (12).

Table 1 Simultaneous determination of GFR and ERPF by means of single injection and standard clearance technique

Patient	GFR (ml/min/1.73 m ²)		ERPF (ml/min/1.73 m ²)	
	Inulin	^{51}Cr -EDTA	PAH	^{125}I -OIH
1 M A	154.6	147.0	815.0	829.0
2 d P F	65.1	65.0	363.0	361.0
3 B A K.	61.9	67.0	282.0	269.0
4 C M	125.0	126.0	720.0	719.0
5 W J	49.5	56.0	221.0	227.0
6 S F	41.0	51.0	271.0	276.0
7 S K.	38.2	41.0	188.0	189.0
8 d P F	28.0	37.0	209.0	211.0
9 N M	19.2	23.0	91.0	93.0
10 B A	130.0	114.0	655.0	639.0
11 L M	19.8	22.0	83.6	86.0
12 B S	142.6	156.0	852.0	830.0
13 d P F	15.5	19.0	92.3	87.0
14 K P	103.5	111.0	396.0	406.0
15 W H	149.0	143.0	798.0	780.0
16 M P	132.0	129.0	478.0	584.0
17 G C	171.0	178.0	784.0	782.0
18 W B	8.0	10.0	16.0	21.0
19 U B	103.5	101.0	496.0	589.0
20 V D	67.2	103.0	672.0	669.0
21 W T	145.6	137.0	—	—
22 D A	143.8	138.0	408.0	414.0
23 W P	106.0	96.0	516.0	557.0
24 S R	42.0	42.0	—	—
25 B A K.	114.0	114.0	491.0	551.0
26 B T	63.0	75.0	460.0	331.0
27 B I	212.0	174.0	840.0	815.0
28 S U	124.0	148.0	—	—

Glomerular filtration rate

The 28 results are shown in Table 1 in chronological order. It can be seen in Fig. 1 that the inulin clearance on the ordinate and the ^51Cr EDTA single injection clearance on the abscissa give a regression line of the form $y = a + b_1 x$ whereby $a = 0$ i.e. $y = 0.991x$ with a standard error of the regression coefficient $s_{b_1} = 0.023$. From the fact that the regression coefficient is almost 1 and its standard error is extremely small it can be concluded that both determinations of GFR are very well in accordance. Table 2a in addition gives the 95% confidence limits for a single future observed ^51Cr EDTA value calculated from the present values with the factor $t_{0.975} = 2.052$.

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25 results are shown in Table 1. In Fig. 2 the PAH-clearance is plotted against ^{125}I OIH giving

COMPARISON OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW DETERMINATIONS OBTAINED BY A SINGLE INJECTION TECHNIQUE AND BY MEANS OF A STANDARD CLEARANCE TECHNIQUE IN CHILDREN

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University of Berne Switzerland*

Single injection techniques to determine glomerular filtration rate and effective renal plasma flow from plasma disappearance curves without urine collections, have repeatedly been studied (1, 2, 3, 8, 9, 11, 13, 14, 15, 17, 18, 21-25). One of the reasons to eventually introduce the single injection method is its simplicity without need of collecting urine. The single injection technique for determination of glomerular filtration rate with ⁵¹Cr-EDTA and of effective renal plasma flow with ¹²⁵I-IOH has been introduced in adult patients in a number of clinics (18, 22). Since further empirical evaluation of the theoretical model (17) is strongly suggested by Truniger et al (22) it was decided to compare the single injection technique, as proposed for children by Donath (4), with a standard clearance technique. The comparison was done by simultaneous determination of glomerular filtration rate and effective renal plasma flow by the two different methods.

MATERIAL AND METHODS

Selection of patients

All boys and girls needing determination of renal function were taken into the study. This comprised infants as well as children up to the age of 16 years. Their functional status ranged from severe insufficiency to high normal values. Only those results of inulin and PAH clearance were accepted for statistical evaluation in which clearances of each period did not differ more than 20 ml/min/1.73 m². The results obtained by the single injection technique were only accepted for evaluation when technical errors had been excluded. An independent investigator performed the statistical comparison. 28 determinations of glomerular filtration rate (GFR) and 25 determinations of effective renal plasma flow (ERPF) will be compared in this study.

Inulin and PAH clearance

After a priming dose of 1 ml/kg 10% inulin and 0.05 ml/kg 20% PAH solution an intravenous sustaining infusion was started containing enough inulin and PAH to maintain a plasma inulin level of 50 mg/100 ml and a PAH level of 2 mg/100 ml. The infusion rate was 1 ml/min/m². After an equilibration period of 45 min three clearance periods of 30 min duration were taken. Repeated venous blood samples were obtained from an indwelling needle that was flushed with a slightly heparinized isotonic saline solution after each sampling.

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inulin and PAH clearances that the isotope method can be regarded to be sufficiently accurate for all practical purposes

The seemingly wide range of the confidence limits for a single future value by means of the single injection technique is due to the relatively small number of cases. Since the regression coefficient for GFR as well as for ERPF is close to 1 and its standard error is extremely small the conclusion of this study is not questioned by this fact

The reference value of the classical clearance technique with continuous intravenous infusion of the clearance substance and urine collection by means of bladder catheterisation is obviously not questioned by this report. This method is however often criticised for its inconvenience for the child and moreover for the risks of bladder catheterisation (5, 15, 18, 20, 22, 25, 26). In addition the chemical determination of inulin and PAH may significantly be altered by the presence of other substances like medicaments (16). The variability of the inulin and PAH-clearances may be as high as 10%. Zander et al. (26) attribute this mainly to the difficulty of collecting urines appropriately. Nevertheless for exact and physiologic studies with determination of special functional parameters the use of the classical clearance methods with urine collection is furthermore indispensable.

If on the other hand only GFR and effective renal plasma flow are asked for the use of the single injection method has its value. No catheterisation of the bladder is necessary. Generally only one vein needs to be punctured. The danger of irradiation is minimal because of the very short biological half-life of the substances. It is less than 60 min for ^{51}Cr EDTA and less than 30 min for ^{125}I IOH when renal function is normal and less than 8 hours and 100 min respectively in advanced renal failure. Thus the total radiation dose is far lower than that of an intravenous urography. This view is well in agreement with the opinion of others (10). The following situations are considered as special

indications for the use of the single injection technique in pediatrics

(a) impossibility of an appropriate collection of urine as in patients with severe oliguria in patients with Bricker bladder or ureterosigmoidostomy and in patients with hydronephrosis or vesico-ureteral reflux

(b) in patients with increased risk of infection by catheterisation for instance after unilateral nephrectomy

SUMMARY

In children 28 values for glomerular filtration rate and 25 values for effective renal plasma flow were simultaneously obtained by means of the inulin and PAH-clearance and by means of a single injection method with ^{51}Cr EDTA and ^{125}I IOH. These were compared in an appropriate statistical model. The results show good agreement of the two methods. The single injection method can therefore be of value in childhood when only values for GFR and effective renal plasma flow are asked or especially if inappropriate urinary collection is expected or bladder catheterisation is contraindicated. It has no place in studies concerned with renal physiology or pathophysiology.

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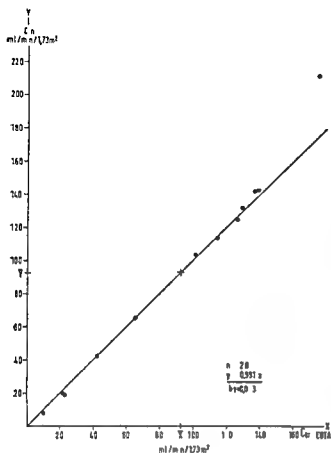


Fig 1 Relation $C_{41Cr-EDTA}$ - C_{Inulin}

ing the regression line $y = 1.007x$ standard error $s_b = 0.007$. The correlation of the two methods is excellent. The 95% confidence limits of a future observed $^{51}Cr-EDTA$ value are shown in Table 2 b. Factor $t_{0.97} = 2.064$.

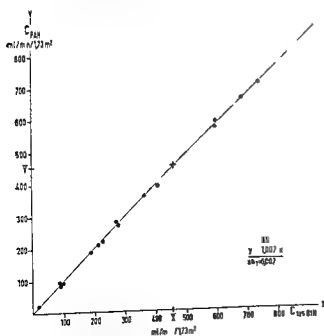


Fig 2 Relation $C_{125I-OIH}$ - C_{PAH}

Table 2 Calculation of the 95% confidence limits for a single future observed value

(a) GFR $x = ^{51}Cr-EDTA$ $y = Inulin$

if $x = 10$ ml/min	$17.67 > y > 2.16$ ml/min
20	30.80 8.84
40	55.13 24.17
60	78.64 40.29
80	101.52 57.07
100	124.06 74.18
120	146.36 91.52
140	168.44 108.08
160	190.48 126.69
180	212.25 146.57

(b) ERPF $x = ^{125}I-OIH$ $y = PAH$

if $x = 50$ ml/min	$61.25 > y > 39.48$ ml/min
100	116.15 85.31
200	223.36 179.56
300	329.13 275.25
400	434.30 371.60
500	538.73 468.57
600	642.97 565.79
700	746.97 663.25
800	850.78 760.91

DISCUSSION

The observation that the regression line passes through the origine ($a=0$) is consistent with the view of earlier investigators (6, 7, 9, 22) that $^{51}Cr-EDTA$ is handled by the kidneys like inulin. They contrast however to some extent with the findings of Stacy et al (19) who considers a possible $^{51}Cr-EDTA$ chelate binding to plasma proteins. This has been refused however by Favre et al (6). Speck (18) has observed that $^{51}Cr-EDTA$ measurements underestimate GFR. The present results are in accordance with this view to some extent in the high normal range (Fig 1), but not in normal and low values.

In order to determine effective renal plasma flow it was crucial to maintain a plasma level around 2 mg/100 ml PAH, since renal excretion of PAH would be incomplete with plasma levels above 3 mg/100 ml (20). This was the reason that three determinations of PAH clearance had to be excluded from the ERPF study (Table 1).

The statistical results show such good correlation between the single injection method for determination of GFR and ERPF with the

inulin and PAH-clearances that the isotope method can be regarded to be sufficiently accurate for all practical purposes

The seemingly wide range of the confidence limits for a single future value by means of the single injection technique is due to the relatively small number of cases. Since the regression coefficient for GFR as well as for ERPF is close to 1 and its standard error is extremely small the conclusion of this study is not questioned by this fact

The reference value of the classical clearance technique with continuous intravenous infusion of the clearance substance and urine collection by means of bladder catheterisation is obviously not questioned by this report. This method is however often criticised for its inconvenience for the child and moreover for the risks of bladder catheterisation (5, 15, 18, 20, 22, 25, 26). In addition the chemical determination of inulin and PAH may significantly be altered by the presence of other substances like medicaments (16). The variability of the inulin and PAH-clearances may be as high as 10% (Zender et al. (26) attribute this mainly to the difficulty of collecting urines appropriately). Nevertheless for exact and physiologic studies with determination of special functional parameters the use of the classical clearance methods with urine collection is furthermore indispensable.

If on the other hand only GFR and effective renal plasma flow are asked for the use of the single injection method has its value. No catheterisation of the bladder is necessary. Generally only one vein needs to be punctured. The danger of irradiation is minimal because of the very short biological half-life of the substances. It is less than 60 min for ^{51}Cr EDTA and less than 30 min for ^{131}I IOH when renal function is normal and less than 6 hours and 100 min respectively in advanced renal failure. Thus the total irradiation dose is far lower than that of an intravenous urography. This view is well in agreement with the opinion of others (10). The following situations are considered as special

indications for the use of the single injection technique in pediatrics

(a) impossibility of an appropriate collection of urine as in patients with severe oliguria in patients with Bricker bladder or ureterosigmoidostomy and in patients with hydronephrosis or vesico-ureteral reflux

(b) in patients with increased risk of infection by catheterisation for instance after unilateral nephrectomy

SUMMARY

In children 28 values for glomerular filtration rate and 25 values for effective renal plasma flow were simultaneously obtained by means of the inulin and PAH-clearance and by means of a single injection method with ^{51}Cr EDTA and ^{131}I IOH. These were compared in an appropriate statistical model. The results show good agreement of the two methods. The single injection method can therefore be of value in childhood when only values for GFR and effective renal plasma flow are asked or especially if inappropriate urinary collection is expected or bladder catheterisation is contraindicated. It has no place in studies concerned with renal physiology or pathophysiology.

ACKNOWLEDGEMENTS

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Key words Glomerular filtration rate effective renal plasma flow injection technique

MALNUTRITION AND SIZE OF THE CEREBRAL VENTRICLES¹

*Echoencephalographic Studies in Infants and Young Children
Preliminary Communication*

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*From the Department of Paediatrics University Hospital Uppsala Sweden
and the Ethiopian Nutrition Institute Addis Ababa Ethiopia*

Severe caloric undernutrition and/or severe protein malnutrition instigated at an early age may cause permanent damage to the brain thereby affecting weight cell number and fine structure as well as biochemistry and function as has been clearly shown from animal experiments (3 8 17 18)

Clinical observations rarely lend themselves to such easy interpretation as well planned animal experiments. Malnutrition at an early age in children of developing countries is often complex in origin (intra and extrauterine influence simultaneous nutrient depletion and infection other ecological factors). Furthermore the possibilities of analysing the human brain with respect to structure and biochemistry are hampered by the fact that biopsy on living subjects cannot be performed. Much of our available knowledge stems from autopsy findings in children dying at varying ages and for reasons of varying complexity. In one respect however follow up studies of human beings who have experienced severe early malnutrition should lend themselves to more detailed interpretation than is possible in animals namely when dealing with complex brain functions as reflected in mental behaviour and intelligence.

But here again evaluation of the effect of a deficient nutrient supply *per se* becomes extremely difficult (2).

It seems obvious that methods which permit an *in vivo* appreciation of the status of the human brain and which allow repeated examinations of a longitudinal character in individual children deserve special interest. Under this heading it has so far been possible to include only head circumference (as an indirect measure of brain volume (13 14)) and transillumination (as an expression of subarachnoidal effusion (6)).

In the following another type of study will be described namely echoencephalography. This method makes possible a determination of the size of the ventricular cavities of the brain and this in combination with the head circumference and transillumination provides more comprehensive information on the brain volume *in vivo*.

The studies presented in this paper were carried out at the Ethiopian Nutrition Institute Addis Ababa. This institute is supported by the Ethiopian government and the Swedish International Development Authority (SIDA) (15).

CLINICAL MATERIAL

A total of 66 Ethiopian infants and children 29 girls and 37 boys of ages 2 weeks to 29 months were examined (Fig 1).

CNU Report no 5.

This work is dedicated to Professor K. H. Schafer Pediatric Clinic Eppendorf Krankenhaus Hamburg on his 60th birthday.

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RESULTS

Weight in relation to age

As can be seen in Fig 4 the body weights in relation to age of all the controls up to 9 months of age lay within—or on the borderline of—the normal ranges (mean ± 2 SD) for Swedish infants (5) In the control children

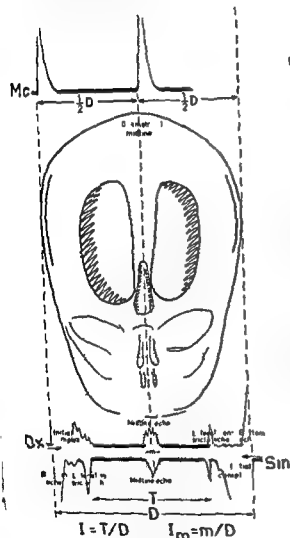


Fig 2 Schematic premo-ran compared with enlarged schematic echoventriculogram (EVG see Fig 3) where *Mc* is the midline control *Dx* the echoencephalogram from the right and *Sin* an inverted echoencephalogram from the left temporal region. The portion of the lateral ventricle echoes correspond to the lateral surface of the lateral ventricles, and the echo-free zones to the widths of the lateral ventricles

EVG

Diagram

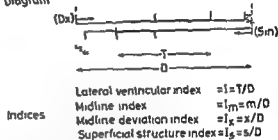


Fig 3 An echoventriculogram (EVG) within the grey field where *Mc* represents the midline control *Dx* an echoencephalogram from the right and *Sin* one from the left temporal region. The graphical representation and index calculation is explained in the text

above 9 months of age the weights lay in the lower part of or somewhat below the normal range for Swedish children

All the marasmic and kwashiorkor patients showed weights far below the normal range with one exception a 7 month old boy with a disproportionately large head he was also the only one of the marasmic patients who had a lateral ventricle index somewhat above the normal range¹

Head circumference in relation to age

Fig 5 shows that 35 of the 38 control children had a head circumference within the normal range according to O'Neill (7) and Westropp & Barber (16) Only 2 children showed values slightly below the lower range and one girl of 19 months of age had a head circumference of 54 cm

This boy developed all classical signs of infantile hydrocephalus during a 2 month follow up period

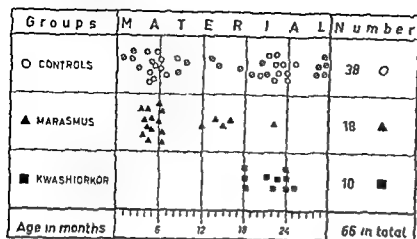


Fig 1 Age distribution of children examined

Control group

This group consisted of 38 children (18 girls and 20 boys) who were apparently healthy and had no history of malnutrition or severe disease. Of these 19 children (8 girls and 11 boys) between 2 weeks and 29 months of age were residents at the Children's Home (marked ○ in the figures) an orphanage attached to the Ethiopian Nutrition Institute and 19 (10 girls and 9 boys) between 4 and 26 months of age belonged to well-to-do and otherwise privileged Ethiopian families (marked ● in the figures). The children at the orphanage were all examined 2 or 3 times. Only the values from the 1st examination are presented.

Marasmic group

Eighteen patients (7 girls and 11 boys) from 3.5 to 22 months of age with confirmed nutritional marasmus were attending either a special Nutrition Rehabilitation Clinic run at the Lidetta MCH Centre (14 children: 6 girls and 8 boys) or the Nutrition Rehabilitation Clinic run by the Ethio-Swedish Pediatric Clinic (4 children: 1 girl and 3 boys). The children were examined twice with an interval of about 1 month. Only values from the last examination are presented.

Kwashiorkor group

Ten patients (4 girls and 6 boys) between 18 and 26 months of age were admitted to the Ethio-Swedish Pediatric Clinic where the diagnosis of kwashiorkor was confirmed. The children in this group were examined twice: first between 1 and 2 weeks after admission—at this time all children were free from visible oedema—and secondly after further 2 weeks. In this case only the values from the first examination are presented.

METHODS

All the children included in the study were examined by the same pediatrician (G.E.). Standard anthropometric data including body weight and head circumference were recorded. In addition the widths of the

lateral ventricles of the brain were measured by echoencephalography according to the method of Sjogren (10).

The echoencephalograph used was a Siemens apparatus with a camera (Polaroid Land) for instant recording of the oscillographic tracings. The probe used in this study had a frequency of 2 megacycles/sec and a diameter of 24 mm. Liquid paraffin was used as contact medium between the head and the probe. The head was not shaved prior to the examination.

The probe was held in contact with the scalp a few centimetres above and slightly anterior to the external auditory meatus. The tracing on the oscilloscope was studied while the probes were moved. Pictures were taken when the midline echo and the lateral ventricle echo on the opposite side were of maximal steepness on the true axis (Fig 2). The amplification was then increased and the distribution of the parenchymal echoes was noted.

Interpretation of the echoencephalograms and index calculations from the echoventriculogram

An echoventriculogram (EVG) consists of a midline control (Mfc) and echoencephalograms from the right (Dr) and left (Sin) sides of the head. Fig 3 illustrates a typical echoventriculogram and diagram of the index calculation.

The geometrical midline of the head and the external diameter (D) in the frontal plane were obtained from the midline control study. From each echoencephalogram the distances from the initial complex to the midline echo, the contralateral ventricle echo and the bottom echo were measured. These distances were measured and marked in the figure from left to right for the Dr echoencephalograms and from right to left for the Sin echoencephalogram.

The lateral ventricle index (I) expresses the relation between the transverse width (T) of the two lateral ventricles and the external diameter (D) of the child's head, i.e. T/D . Normally this index should not exceed 0.33 (or 33% of the external diameter of the head) in newborn babies. In children of 1 year of age or more the lateral ventricle index should not exceed 0.29.

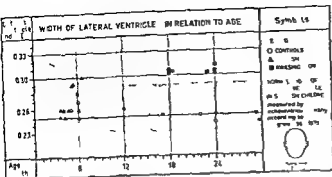


Fig 6 Width of lateral ventricles in relation to age

brain weights at autopsy were found to be normal for the age. In contrast to these findings Monckeberg (6) observed increased transillumination in infants with marasmus pointing to more pronounced stunting of the brain growth than was indicated by head circumference measurements alone in the same patients.

In children dying with a disease picture of severe marasmus Winick & Rosso (19) found a close correlation between head circumference and DNA content of the brain thus indicating that the head circumference may be a good index of the total number of brain cells. The number of children examined was limited however.

In view of the conflicting reports it seemed important to scrutinize further the relationship between head circumference and brain size. An increase in volume of the cavities in the interior of the brain and/or of the fluid layer on the exterior of the brain wall diminish the size of the brain substance in relation to the head circumference. The same is true for an increase in cranial thickness (soft tissue included). A diminution of the inner cavities and/or a reduction of the skull thickness will have the opposite effect.

Echoencephalography makes it possible to perform accurate and repeated determinations of the widths of the lateral ventricles with no discomfort or harm to the child. A comparison of the findings in echoencephalography with those of pneumo-encephalography has shown a good correlation (11). The present study showed in the children with kwashiorkor examined 1-2

weeks after admission when oedema was no longer visible a slight but significant increase of the widths of the lateral ventricles. This deviation from the normal was still found to be present to the same extent at reexamination 2 weeks later. The children with marasmus on the other hand showed values within the normal range when examined twice with an interval of 1 month.

Are there any sources of error which might render invalid the interpretation of these results when comparing them with those obtained in healthy children? Transillumination studies in our series so far performed only in children with kwashiorkor showed moderately but definitely increased values. However even if these latter findings can be interpreted in the usual way as an expression of increased fluid between the brain surface and the skull (which is perhaps not truly valid until the influence of bone thickness and structure and of soft tissue oedema etc. has been further elucidated) this will not compromise the finding of an increased T/D value in kwashiorkor children as an expression of relatively increased ventricular widths.

One source of error in studies in a developing country relates to difficulties in obtaining an exact age. For children in the first few years of life the error is usually not great and furthermore the ventricular index as seen in Fig 6 does not change markedly with age after the first 6 months of life.

The reason why the marasmic infants and children showed a normal ventricular index whereas those with kwashiorkor showed an in-

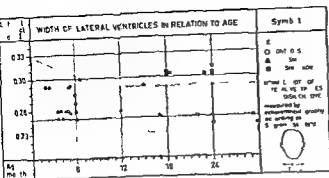


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The reason why the marasmic infants and children showed a normal ventricular index whereas those with kwashiorkor showed an in-

crease is not clear. It cannot be explained by the difference in age alone, since the findings in the marasmic children of similar age to those with kwashiorkor were the same as in the young infants with marasmus.

The body weight of the kwashiorkor children was in most cases markedly below the lower normal limit. This might indicate that a prolonged period of protein-calorie malnutrition had preceded the onset of the kwashiorkor symptoms.

Further studies with repeated examination of the ventricular index in individual children will give an answer to the question of whether the increased ventricular index observed in children with kwashiorkor up to 3-4 weeks after initiation of treatment is a temporary phenomenon or not. Such studies are now under way.

They also include parallel transillumination examinations and studies of the nerve conduction velocity, as well as scorings of the psychosocial and motor ages of the children.

SUMMARY

Examination of the size of the cerebral ventricles by means of echoencephalography in Ethiopian infants and young children with severe malnutrition showed a moderate but significant increase in children with kwashiorkor, examined up to 3-4 weeks after admission, whereas children with marasmus showed no deviation from the normal.

Further studies will show whether or not this abnormality in the kwashiorkor children is a transient phenomenon. The observation gives one more reason for caution in using head circumference as a measure of brain size.

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The authors are indebted to the staff of the Ethio-Swedish Pediatric Clinic (Head Yngve Larsson) and of the Lidetta clinic (Head Ulla Larsson) for valuable cooperation in the selection of patients for this study.

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HIGH FREQUENCY OF SALMONELLA SPECIES AS A CAUSE OF NEONATAL MENINGITIS IN IBADAN, NIGERIA

A Review of Thirty eight Cases

NAOMI BARCLAY

From the Department of Paediatrics University College Hospital Ibadan Nigeria

It is well known that gram negative enterobacteria account for a substantial proportion of the cases of meningitis occurring in early infancy (13, 16, 23). However, apart from three extensive reviews in 1946, 1951, 1958 there has been a notable silence on cases of meningitis due to *salmonella* species, and since 1958 only 33 cases have been reported.

Analysis of cases of infantile meningitis in Ibadan showed a high incidence of *salmonella* infections, many of which were resistant to the standard antibiotics.

PATIENTS AND METHODS

All cases of *salmonella* meningitis admitted to the University College Hospital Ibadan between January 1961 and May 1969 were reviewed. 508 other cases of bacterial meningitis (excluding tuberculous) admitted between January 1964 and May 1969 were studied for comparison. Nine patients with abnor-

malities of the central nervous system were excluded. An analysis of the pattern of antibiotic sensitivity of *salmonella* isolated from blood cultures over the same period was also made.

Once a diagnosis of meningitis was decided on all the children received penicillin (20 000 units/kg/24 h) and chloramphenicol (50-100 mg/kg/24 h) usually intravenously with the addition of sulphadiazine (200 mg/kg/24 h) by the same route except in neonatal or dehydrated patients. No intrathecal antibiotics were administered. Therapy was modified as necessary as bacteriological sensitivities became available.

RESULTS

The pneumococcus was the major overall cause of meningitis. *salmonella* species were the commonest enterobacteria.

Pneumococcus accounted for 184 patients, half of whom were over 1 year of age, and many of whom were adults. Throughout the first year of life (Fig. 1) *haemophilus influenzae* and gram negative enterobacteria were

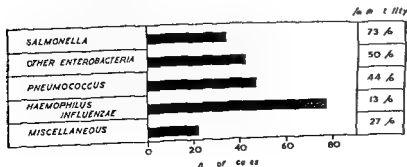


Fig. 1. Organisms causing meningitis under 1 year of age in Ibadan. 269 cases.

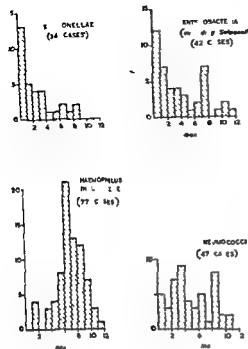


Fig 2 Age distribution of the different meningitides in Ibadan

the predominant organisms isolated Fig 2 illustrates the age distribution of the different types

Cases caused by salmonella species were similarly distributed to those caused by the other enterobacteria one third of these occurred in the first month of life during which period salmonella species was the major cause of meningitis (Fig 3) O-typing was done in 10 instances. The strains identified were

Salmonella G and B 3 cases each
Salmonella A E E₁ 1 case each
Salmonella typhimurium 1 case

Table 1 Organisms comprising the miscellaneous group of meningitides in Ibadan

	Under 1 y	Over 1 y	Total
Staph. Pyogenes	10	8	18
Meningococcus	2	8	10
Haem. parainfluenza	3	2	5
Haemolytic streptococcus	0	3	3
Strep. viridans	4	0	4
Strep. faecalis	1	0	1
Total	20	21	41

Other enterobacteria isolated are indicated on Table 2

The miscellaneous organisms encountered are shown in Table 1. In the remaining 91 cases (44 of which were under 1 year old) no organisms were isolated but owing to the method of selection of cases this figure represents a considerable underestimate.

Salmonella meningitis

The most significant finding was the high proportion of salmonella species which were resistant not only to chloramphenicol but also to tetracycline, streptomycin and the sulphonamides. Between 1964 and 1966 there were only two cases of salmonella meningitis showing resistance to these antibiotics at the UCH and a similar pattern was reflected amongst salmonellae causing bacteraemia. These proportions have increased dramatically over the past 5 years. In 1969 for instance there was only one chloramphenicol sensitive case out of the 10 salmonella meningitides and 38 out of 80 cases of salmonella bacteraemia. Salmonella typhi on the other hand has re-

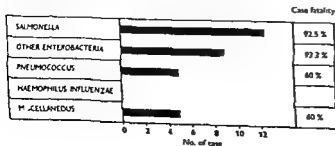


Fig 3 Causative organisms involved in neonatal meningitis in Ibadan 43 cases.

HIGH FREQUENCY OF SALMONELLA SPECIES AS A CAUSE OF NEONATAL MENINGITIS IN IBADAN, NIGERIA

A Review of Thirty eight Cases

NAOMI BARCLAY

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It is well known that gram negative enterobacteria account for a substantial proportion of the cases of meningitis occurring in early infancy (13, 16, 23). However apart from three extensive reviews in 1946, 1951, 1958 there has been a notable silence on cases of meningitis due to salmonella species, and since 1958 only 33 cases have been reported.

Analysis of cases of infantile meningitis in Ibadan showed a high incidence of salmonella infections, many of which were resistant to the standard antibiotics.

PATIENTS AND METHODS

All cases of salmonella meningitis admitted to the University College Hospital Ibadan between January 1961 and May 1969 were reviewed. 508 other cases of bacterial meningitis (excluding tuberculous) admitted between January 1964 and May 1969 were studied for comparison. Nine patients with abnor-

malities of the central nervous system were excluded. An analysis of the pattern of antibiotic sensitivity of salmonella isolated from blood cultures over the same period was also made.

Once a diagnosis of meningitis was decided on all the children received penicillin (20 000 units/kg/24 h) and chloramphenicol (50-100 mg/kg/24 h) usually intravenously with the addition of sulphadiazine (200 mg/kg/24 h) by the same route except in neonatal or dehydrated patients. No intrathecal antibiotics were administered. Therapy was modified as necessary as bacteriological sensitivities became available.

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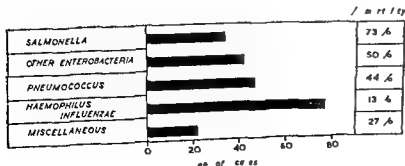


Fig 1 Organisms causing meningitis under 1 year of age in Ibadan 269 cases

Table 3 *Salmonella meningitis* Review of literature

Author	Year	Source	Neonates		1 month + mixed		Total	Case fatality (%)
			Cases	Died	Cases	Died	Cases	
Henderson	1948	Review	49	48	98		147	96
Beene	1951	Review (+1 new)			88	72	88	84
Watson K C	1957	Durban (4 + review)	55	52	87	69	142	85.5
Reports in literature	1958-1968		2		31*		33	
Present series	1969	Ibadan	14	13	24	16	38	76

* Several described as infants

have a high case fatality *per se* (5.9-23.25) in Ibadan there is in addition a resistance to antibiotics that has arisen in all groups of enterobacteria and these account for 50% of all neonatal cases of meningitis. Since there is nothing specific about the symptoms of enterobacterial infection in infants and the fatality is high the importance of early and energetic treatment aimed at these resistant strains cannot be over-emphasised.

Antibiotic resistance of salmonella species has only been reported once previously (11) and one hopes that this lack of resistance is as real as it is apparent.

SUMMARY

538 cases of bacterial meningitis admitted from January 1964 to May 1969 in all age groups at the UCH Ibadan were reviewed. The proportion of enterobacterial cases of meningitis admitted to the UCH Ibadan was higher than has been reported from any other centre the maximum incidence being in the neonatal period. A very large number of these

were due to various salmonella species and 70% of these are currently resistant to the more commonly used antibiotics including chloramphenicol. This resistance to chloramphenicol is reflected amongst all enterobacteria isolated in Ibadan and poses a considerable problem. At present over 90% of neonates with enterobacterial meningitis die and this case fatality is maintained in the older age groups in cases where resistant strains are encountered.

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Table 4 Delay in appropriate therapy related to mortality

Delay (days)	Alive	Dead	Total
0-7	8	6	14
8-14	1	4	5
15-21	0	5	5
22	0	2	2
Total			26

Table 2 *Enterobacterial meningitis causative organisms*

Author	E coli	Para colon	Proteus	Pseudo monas	Kleb siella	Salmo nella	B alk ligenes	Aero bacter	Unidenti fied	Total
Previous literature	111	27	26	13	4	10	1	12	14	218
Present series	9	2	3	6	7	35	—	—	40	102
Total	120	29	29	19	11	45	1	12	54	320

maintained consistently sensitive to both tetracycline and chloramphenicol. An analysis of 59 non-salmonella enterobacterial meningitides showed a similar trend (46% resistant), illustrated in Fig. 4.

Salmonella meningitis as seen in Ibadan is predominantly a disease of neonates and young infants. Only 2 out of 38 patients developed the disease over the age of 1 year, at 18 months and 3 years respectively. 20 were males, 16 were females, the sex being unrecorded in two. There was neither seasonal variation nor evidence of an epidemic pattern. One child developed meningitis two weeks after admission for prematurity, and three patients were transferred from the Ibadan University Rural Health Training Centre, 60 miles away, but most patients were admitted directly to the UCH.

The case fatality for salmonella meningitis was high in all age groups, 73.5% overall and 92.5% in the neonatal patients. Five children developed subdural effusions and two of these died. One child's meningitis developed second-

ary to an untreated otitis media, there was no obvious focus of infection in the rest.

Resistance of the organism to chloramphenicol appeared to increase the likelihood of a fatal outcome, but this proved to be an artefact due to a close association with delay in treatment.

DISCUSSION

Most reports of enterobacterial meningitis in infants have come from the United States or Europe (Table 2). It has not so far been recognised as a significant problem in the tropics or developing countries. Geethuysen (1960), Pirame (1968) and Senecal (1957), reporting on large series gave relative incidences of enterobacterial meningitis compared to other types as 2%, 5%, 3% and 0% respectively. Previous reports from Nigeria have shown similar figures (1, 14).

The first report of salmonella meningitis (as quoted by Mpairwe, 1968) was made by Ghon in 1908. By 1948, Henderson had collected 147 cases from the world literature. Beene, Hansen & Fulton reviewed 87 recent case reports in 1951. Watson collected a further 142 cases in 1958 (Table 3).

This is the largest number of new cases that have been reported from one institution, indicating that, in the period studied, salmonella meningitis is probably markedly more common in Ibadan than in other centres.

The most significant prognostic factor was delay in institution of appropriate antibiotic treatment after the onset of symptoms ($p < 0.001$). This is clearly shown in Table 4.

Although neonatal meningitis is known to

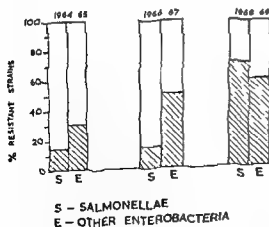


Fig. 4 Increase in resistant strains of enterobacteria over the past 5 years

HEMISPHERECTOMY IN EARLY HEMIPLEGIA AND INTRACTABLE EPILEPSY

T. TÖRMÄ and MARTA DONNER

*From the Neurosurgical Department of the University Central Hospital of Turku
and the Childrens Clinic the University Central Hospital of Helsinki Finland*

When infantile hemiplegia is associated with severe epilepsy and serious behaviour disturbances interfering with normal adjustment at home and in school hemispherectomy may be contemplated. This therapy is based on the assumptions that in cases of early injury the other cerebral hemisphere in part at least assumes the functions of the affected side and that function on the intact side is disturbed by the pathological activity in the affected hemisphere: the frequent convulsions and massive medication. In cases of this kind hemispherectomy has been used for therapeutic purposes since the 1930s. The method was first mentioned by McKenzie (11) but the chief pioneer was Krynauw (7) who reported 12 cases. Since then over 400 cases have been treated by hemispherectomy (reviews by White (14) and Ignelzi & Bucy (5)). The results have been very gratifying in a large number of cases but only in a minor proportion can the effect be evaluated on the basis of observations covering many years. For this reason we feel justified in presenting 6 patients treated by hemispherectomy who have been followed for 3 to 11 years.

The following indications for operation have been mentioned in the literature:

1) early hemiplegia 2) epilepsy refractory to ordinary doses of antiepileptic drugs 3) serious behaviour disturbances interfering with social

adjustment 4) progressive impairment of intellectual performance 5) pneumoencephalographic findings indicative of unilateral involvement.

As a rule a combination of all or most of these indications has been regarded as necessary.

In the majority of reported cases hemiplegia has had its origin pre- or perinatally or postnatally in infancy or very early childhood. It has not been possible to define any definite upper age limit however and the operative series comprises cases in which hemiplegia developed at school age. Besides hemiplegia the main indication has invariably been frequent convulsive fits refractory to ordinary therapy. The behaviour disturbances have mostly consisted of severe restlessness, hyperkinesia and aggressiveness. Hemiplegias due to degenerative brain disorders are contraindications to hemispherectomy. On the other hand increasing dementia caused by anoxic injuries during severe seizures and excessive anticonvulsant medication are important indications. It is considered important that the pneumoencephalographic findings are largely limited to one hemisphere. They consist of dilatation of the lateral ventricle on the affected side and a shift of the midline towards this side (7). The electroencephalogram does not have the same prognostic value. Although patients with

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The following indications for operation have been mentioned in the literature:

1) early hemiplegia 2) epilepsy refractory to ordinary doses of antiepileptic drugs 3) serious behaviour disturbances interfering with social

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In the majority of reported cases hemiplegia has had its origin pre- or perinatally or postnatally in infancy or very early childhood. It has not been possible to define any definite upper age limit however and the operative series comprises cases in which hemiplegia developed at school age. Besides hemiplegia the main indication has invariably been frequent convulsive fits refractory to ordinary therapy. The behaviour disturbances have mostly consisted of severe restlessness, hyperkinesia and aggressiveness. Hemiplegias due to degenerative brain disorders are contraindications to hemispherectomy. On the other hand increasing dementia caused by anoxic injuries during severe seizures and excessive anticonvulsant medication are important indications. It is considered important that the pneumoencephalographic findings are largely limited to one hemisphere. They consist of dilatation of the lateral ventricle on the affected side and a shift of the midline towards this side (7). The electroencephalogram does not have the same prognostic value. Although patients with

unilateral changes seem to have the best prognosis (13) bilateral changes of the background activity and bilateral spikes and waves do not preclude good results (7)

The surgical technique varies to some degree. Part of the temporal lobe is left by some surgeons while the majority today excise the entire lobe. The incision through the region of the basal ganglia is now perhaps most often made between the nucleus caudatus, most of which is excised, and the thalamus, which is left for the most part (9). The operative mortality has ranged from 5 to 10%.

A good or satisfactory result has been reported in the vast majority of cases. As a rule the hemiplegia is transiently impaired. Initially a flaccid paresis results but usually the end result is reduced spasticity and a wider range of motion than preoperatively. The drawback is a loss of thumb capacity for opposition and circumduction, finger capacity for piano movements and hand capacity for supination as well as impairment of sensation (8). The epileptic attacks are completely abolished in two thirds of the patients and alleviated in at least half of the remainder. Largely similar results have been noted in regard to the behaviour disturbances. Neither behaviour nor epilepsy have been adversely affected by the operation (4).

Postoperative improvement in their IQ scores has been reported for many patients (10). The improvement is probably due to increased mental efficiency and better concentration. A decrease in the IQ scores has been noted in about 10% of cases.

As an additional handicap homonymous hemianopsia develops in all cases in which it was not already present.

On the other hand the most dreaded kind of additional disability, i.e. aphasia resulting from excision of the dominant hemisphere has not proved important (1). Transient aphasia has been reported in a minor proportion of the cases mainly in those in which hemiplegia developed after the patient started talking but equally often in association with right sided as

with left sided hemispherectomy. In some patients the development of speech was accelerated after the operation.

During the last few years the enthusiasm for hemispherectomy has decreased owing to the late complications reported in some operative series (2, 3, 12). These complications originate in a cyst formed at the site of the excised hemisphere. The cyst may be separated from the remainder of the subarachnoid space, with local expansion or hydrocephalus resulting or there may develop in its wall a granulating often bleeding membrane resembling that seen in subdural haematoma. The bleeding causes haemosiderosis and irritation of the ependyma and meninges, leading to the formation of adhesions and hydrocephalus. These complications may occur as much as 6 to 12 years postoperatively, and it is believed that they may occur in up to one fourth of the cases.

MATERIAL

The present series consists of 3 girls and 3 boys aged 4 to 17 years at operation (see Table 1). In 4 cases the cerebral lesion and the spastic hemiplegia were probably due to birth injury. In 2 of these patients the condition deteriorated markedly in connection with a protracted epileptic fit at the age of 3 and 5 years respectively. One girl had an intracerebral haemorrhage at the age of 1 year probably caused by rupture of an aneurysm in the communications on the right. In one case the etiology is unknown but it seems possible that encephalitis was involved. The left hemisphere was involved in 4 cases, the right hemisphere in 2. Convulsive attacks were the main indication for operation in all cases. In spite of anticonvulsant medication for many years the fits had increased in frequency until they occurred many times a day or even many times within an hour. Various types of partial seizures were represented as a rule with secondary generalization.

Five of the 6 patients showed behaviour disturbances. In 3 cases these were so serious that school attendance and continued care at home had become impossible (hyperkinesia, aggressiveness, destructibility). All of these 5 patients showed steady impairment of intellect. In 3 cases the preoperative IQ had dropped to the level of moderate to severe mental retardation (Fig. 1). One boy was too restless to take a psychological test before the operation.

The pneumoencephalographic finding was pathological in all cases. Four showed the picture which is optimal from the standpoint of the result of hemispherectomy, i.e. dilatation of one lateral ven-

Table 1 Six cases of hemispherectomy preoperative data

Case no	Sex	Age at operation (yrs)	Hemiplegia		Epilepsy type and frequency of attacks	Behaviour	Intelligence		Pneumoencephalography
			Age at onset	Cause			Age (yrs)	IQ	
1	♀	10	5 yrs	Encephalitis	Partial and generalized motor many daily	Hyperkinetic aggressive	6 9	106 74	Dilated right ventricle shift to right
2	♂	8	1 yr	Rupture of aneurysm	Partial motor 2-3 daily	Hyperkinetic noisy uncontroll	2 5 8	105 90 57	Dilated right ventricle shift to right
3	♂	17	First months	Birth injury	Partial motor and aphasic up to 10 daily	Not abnormal	13	49	Dilated left ventricle shift to left
4	♀	11	First months deteriorated at 5 yrs	Birth injury & injury during epileptic fit	Partial and generalized motor many daily	Restless aggressive	7 11	49 severely demented	Especially left ventricle but also right and third ventricles and basal cisterns dilated
5	♂	4	First months deteriorated at 3 yrs	Birth injury & injury during epileptic fit	Partial and generalized temporal absences up to 20 per hour	Hyperkinetic, aggressive destructive	Not tested (severe restlessness)		Dilated left ventricle
6	♂	13	First months	Birth injury	Partial and generalized many daily	Restless aggressive	8 10 13	78 72 30	Severely dilated left ventricle right and third ventricle and basal cisterns somewhat dilated

tricle without enlargement of the other ventricle or of the cisterns. Three of these patients showed a lateral shift of the midline of over 5 mm towards the affected side (Fig 2). In the other two cases there was also slight dilatation of the other lateral

ventricle, the third ventricle and the basal cisterns (Fig 3).

In all patients but one the electroencephalogram showed a disturbance of the background activity of both hemispheres and spike-wave paroxysms which



Fig 1 The intelligence quotient (IQ) before and after hemispherectomy



Fig. 2 Case 1 PEG findings before hemispherectomy, enlarged right ventricle (the distance from septum pellucidum to nucleus caudatus 17 mm at the right and 12 mm at the left) Shift to the right of the middle structures (7 mm) Other ventricles and cisterns of normal size

often were generalized. Asymmetry was seen in all patients; however, and in one case the spikes and spike wave complexes were unilateral only.

TREATMENT

The same operative technique was employed in all cases. It has previously been described (6)



Fig. 3 Case 4 PEG findings before the operation. Both ventricles dilated, the left more (Evans index 0.40 at the left and 0.32 at the right) Shift to the

Acta Paediat Scand 60

and does not deviate from the method described in the literature.

In one case (No. 2) a postoperative complication occurred in the form of bleeding from a venous sinus which caused a haematoma entirely filling the operative cavity. After craniotomy and excision of the haematoma, this patient also recovered, although more slowly than the remainder.

Postoperatively all patients initially showed massive contralateral hemiplegia. In this stage none of them had any epileptic fits. During the immediate postoperative stage all the patients showed aggressiveness towards the nursing staff. Five children were transferred at an early stage to a pediatric hospital with good possibilities for rehabilitation, where the anticonvulsant therapy was successively modified and reduced. Patient No. 6 returned home after a short stay at an institution for retarded children. In this case rehabilitation was inadequate.

FOLLOW-UP INVESTIGATION

The interval between hemispherectomy and follow-up investigation ranged from 3 to 8 years. The patients' ages at follow-up ranged from 8 to 25 years (Table 2). In addition to



left of the middle structures (3 mm). The third ventricle is dilated (horizontal diameter 11 mm). Basal cisterns dilated.

Table 2 Six cases of hemispherectomy: postoperative data

Case	Age at follow-up exam (yrs)	Duration of follow-up (yrs)	Side of oper	Complications	Function of arm and leg	Epileptic fits	Intelligence school work or care	Behaviour	Disturbance of vision	Dysphasia
2	11	8	Right	Ostitis at site of oper	Arm not used walks with slight limp	Stopped (5 yrs later some automatic attacks)	Improved IQ 91 finished primary school attends peoples high school	Cheerful euphone passive concentrates well	Homonymous hemianopia	None
1	17	8	Right	Early post oper intracranial bleeding	Arm not used, slight limp	Stopped	Slight deterioration IQ 40 centre for day care	Improved lively unconcentrated	Only half of right field intact	None
3	5	8	Left	—	Arm not used slight limp	Stopped	Improved IQ 77 shop-assistant	Normal (as before operation)	Homonymous hemianopia	Same degree and type as before operation
4	15	4	Left	—	Arm not used slight limp	Less severe fewer attacks	Slight deterioration IQ 30 institutional care	Improved cheerful passive	Homonymous hemianopia	None
5	8	3	Left	Transient respiratory & cardiac arrest 3 months after oper	Arm not used does not walk after complete	Stopped ? small temporal absences 3 yrs after oper	IQ 68 class for subnormal CP children	Improved passive cheerful concentrates	Only half of right field intact	None
6	16	3	Left	Periodical headache	Arm not used walks with difficulty	Only transient alleviation	Further deterioration IQ 0 cared for at home	Improved passive apathetic	Homonymous hemianopia	Difficult to assess

the above mentioned severe complication in the acute stage in case 2 a severe complication also occurred in another patient (No 5) 3 months after operation. The primary post operative result was very good in this case but after 3 months this boy developed pressure symptoms which within 3 weeks led to respiratory and cardiac arrest, necessitating protracted respirator treatment. No drainage was applied. It may be assumed that the condition was due to blocking of the outflow from the

cyst at the site of the excised hemisphere resulting in expansion. In these 2 cases the results were impaired by the complications. In addition one patient (No 1) showed protracted ostitis in the operative area and another patient (No 6) experienced frequent episodes of headache during the first 2 years.

After hemispherectomy 3 patients started walking within a few weeks while 2 patients with a low IQ and that girl who had a post operative haemorrhage did not walk until 1 to

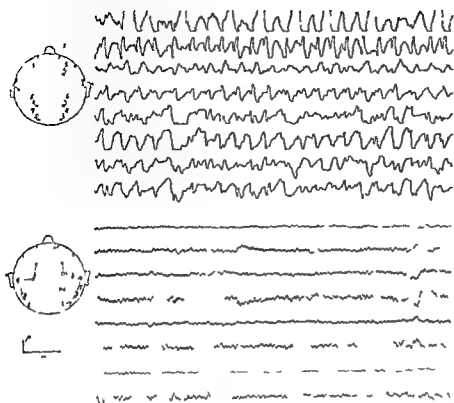


Fig 4 Case 1 EEG registered before the operation and 8 years after the operation (the most pathological segments have been chosen)

2 years postoperatively. The postoperatively occurring severe complication caused worsening of the handicap in case No 5. After this he has not been able to walk without help. None of the 6 patients is able to use the hemiplegic hand which is kept closed with the arm flexed in adduction. In at least 3 cases the sense and stereognostic ability on the hemiplegic side is poorer than before operation. Sensation in all modalities is diminished, and one patient sustains frostbite in the hand without noticing.

The epileptic fits were immediately abolished in 4 cases. Later one of these patients has had brief partial seizures with motor and autonomic symptoms during a short period and another has experienced temporary absences of short duration. In one patient the fits were alleviated while in another (No 6) only transient improvement of short duration was noted and the epilepsy is now as severe as before operation. The electroencephalogram in all cases showed markedly reduced activity on the side of the operation while the background activity on the contralateral side was normal in 3 cases and slightly pathological in 3. Spike-wave paroxysms occur in those 2 cases in

which attacks have persisted. The other 4 patients show occasional sharp transients only (Fig 4).

Two patients show significant improvement in their IQ scores while 3 show a decrease (Fig 1). This group consists of those 2 patients in whom seizures have persisted and that girl who had severe postoperative bleeding. One patient (No 5) could not be tested until after the hemispherectomy. In spite of the above mentioned severe complication involving respiratory failure and further disablement, he has now an IQ of about 60-70. The behaviour of our patients has radically changed for the better in that restlessness and aggressiveness have disappeared. They have become cheerful and calm and better able to concentrate on their tasks than before. Excessive passiveness is now the greatest problem. One patient showed no disturbances of behaviour before operation nor after and one patient is still restless though not to the same extent as restrained and aggressive as preoperatively.

Four patients show homonymous hemianopsia but otherwise their vision is good. In 2 patients one eye is almost blind in one

owing to the primary disorder in the other (No 5) as a result of complicating high intra cranial pressure. Thus these 2 patients can only use half the visual field of one eye. A girl aged 18 who shows the best result of hemispherectomy generally speaking states that the hemianopsia and disturbance of sensation in the hand are now her chief complaints.

Speech has not been impaired in any of our patients although 4 of them were subjected to left sided hemispherectomy. In that patient who has the lowest IQ it is difficult to evaluate the result in this regard however.

Two of our patients were able to attend school after the hemispherectomy and have passed primary school. One of them then received occupational training and is working as a shop assistant. The other one is aiming at further studies in an adult continuation school after 1 year's attendance at evening courses. One child attends a class for subnormal CP children while 3 patients are moderately or severely mentally retarded. Two of these are cared for at home and one at an institution for mentally retarded where she works at the loom.

CONCLUSION

We thus have 2 satisfactory cases (Nos 1 and 3) and one in which the operation was of no benefit (No 6). In 3 cases the result is neither altogether satisfactory nor poor. Two of these patients have a low IQ but one of them (No 2) has been entirely relieved from epileptic fits and the other (No 4) from aggressiveness and restlessness although epilepsy persists. In case No 5 the primary result was good but additional disablement was caused by a late complication.

Considering that those 2 of our 6 patients in whom pneumoencephalography showed signs of more extensive damage had the poorest prognosis (these two did not have complications) we would not recommend hemispherectomy if the pneumoencephalographic findings are not strictly unilateral. Severe mental re-

tardation is also a contraindication as the rehabilitation of these patients after the operation is very difficult. The occurrence of complications many years after operation reported by many authors since 1966 is also an argument for cautiousness.

SUMMARY

Six children and young persons are described in whom hemispherectomy was performed because of intractable epilepsy and in 5 severe behaviour disturbance. All of them had spastic hemiplegia and progressive impairment of the intellectual function before the operation. The follow up period was 3 to 8 years. In two cases the results were satisfactory in 3 cases only partly so. Two of these had severe complications one immediately after the operation and one some months later. The complications probably impaired the results. One boy did not benefit from the operation.

Addendum Recently Wilson has published an extensive report on the early and late results of hemispherectomy. Wilson P J E. Cerebral hemispherectomy for infantile hemiplegia. A report of 50 cases. *Brain* 73 147 1970.

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ORAL D-PENICILLAMINE AND INTRAMUSCULAR BAL+EDTA IN THE TREATMENT OF LEAD ACCUMULATION

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While symptomatic lead poisoning continues to be a problem, the presence of abnormally high concentrations of lead in the tissues of asymptomatic children may have subtle but disastrous effects. Among others, Moncrieff et al (9) and Gibson et al (5) demonstrated the presence of lead accumulation in many retardates. Although this work cannot be fully assessed at the present time, it raises the possibility that unsuspected lead accumulation may be a significant etiological factor in the retardation of otherwise asymptomatic children. In addition, many asymptomatic children with elevated lead levels may go on to develop overt plumbism. These factors make chelating therapy mandatory.

Several substances have been used in treatment. While the combination of BAL and EDTA has been shown to be an efficient chelating regimen, it must be given parenterally and there are a number of side reactions (1, 8). It is therefore recommended in the literature only for severe acute lead encephalopathy (1, 3). D-penicillamine is an effective lead chelator when given orally and is usually well tolerated (1, 6, 10).

In the present study, the effects of these two therapies were compared in 3 children with demonstrated lead accumulation. D-penicillamine

appeared to delead as effectively as BAL and EDTA.

CASE REPORTS

Case 1 J. H. (58/32/13), a 34-month-old Negro girl, had been found eating paint from a paint can 2 months prior to admission. She had also been noted to peel and ingest cracked paint from the walls. A blood lead determination 1 month before admission was 80 µg per 100 ml.

At the time of admission, she was found to be an alert, cheerful and healthy-looking girl. There were no ophthalmological or neurological abnormalities and the examination was unremarkable. Her height was 92 cm (50th percentile) and weight 13.8 kg (40th percentile). Psychological testing determined that she was functioning at the average mental level for her age.

An EEG was normal. Radiological survey showed dense zones of subepiphyseal sclerosis in the distal femora, proximal tibiae and distal long bones of the wrists. Abdominal radiograms revealed a few areas of density in the sigmoid.

There was minimal proteinuria, glucosuria and generalized aminoaciduria; the patterns of urine amino acids varied throughout the course of treatment. Additional laboratory data are given in Table 1.

Case 2 M. O. (63/05/71), a 25-month-old Puerto Rican girl undergoing treatment for ascariasis, was found by a visiting nurse to be eating paint chips from the walls of her home. The blood lead was 100 µg per 100 ml and the patient was admitted for study.

Physical examination revealed a girl who appeared to be anemic. Her height was 83 cm (60th percentile) and her weight 12.7 kg (10th percentile). The rest of the physical examination, including neurological evaluation, was entirely negative. Her developmental evaluation was consistent with her age.

An EEG was within normal limits. Radiological survey of the epiphyses showed very dense wide subepiphyseal bands of sclerosis. No radiopaque densities were seen in the bowel.

The patients were studied in the Yale University Children's Clinical Research Center supported by Public Health Service Grant FR-00125, also partially supported by the Public Health Service (TI AM 5351).

Table 1 Laboratory data

	Patient 1								Patient 2							
	Adm	I	II	III	IV	VI	VII	VIII	Adm	I	II	III	IV	VI	VII	VIII
<i>Blood</i>																
Hemoglobin (g/100 ml)	11.9	11.9	11.1	11.5	11.6	11.9	10.6	10.5	10.7	10.9	11.5	9.7	10.0	9.0	9.1	7.9
Reticulocytes ()		0.9	1.2	2.4	3									4.0		
WBC ($\times 10^3/\text{mm}^3$)	6.1			6.6	8.5	5.6	5.7	4.8	11.2	6.6	10.1		7.5	8.0	8.2	5.5
Neutrophils ()	46				71	15	40	59	30	33	17		43	26	53	34
Lymphocytes ()	43				27	74	50	35	59	57	55		41	56	35	50
Eosinophils ()						5	4	4	10	7	20		4	14	5	12
Bilirubin (mg/100 ml)	0.6							0.5		0.4			0.3			0.8
SGOT (k U)	55							107		27			37			10 ³
Thymol flocc	0							0		0			0			0
Cephalin flocc	0.0							0.0		0.0			0.0			0.3
Thymol turbidity								1		2			2			2
Alk Phos (B U)	13							4		34			15			7
<i>CSF</i>																
Lead ($\mu\text{g}/100 \text{ ml}$)											60		10			
WBC	0										1		1			0
Protein (mg/100 ml)	16										32		20			20

Aminoaciduria of no specific pattern was noted throughout hospitalization. Additional laboratory data are given in Table 1.

Case 3 D C (64 92 26) a 9 year old Puerto Rican boy was one of a large family of children who had been treated for plumbism. A visiting nurse referred him to the clinic where a blood level of 160 μg per 100 ml was found and he was admitted for study.

He was a short shy pale boy. The height of 100 cm and the weight of 10.9 kg were both below the third percentile. No ocular abnormalities were found and except for bilateral positive Babinski signs the remainder of the physical examination was normal. The developmental evaluation placed him in the educable range.

An EEG demonstrated findings suggestive of some disorganization of the normal faster rhythms during the waking state. The findings were diffuse and compatible with some diffuse cerebral dysfunction.

X-rays of the hands revealed some epiphyseal sclerosis and growth lines in the distal forearm while the knees manifested multiple growth lines and accentuation of the subepiphyseal sclerosis consistent with heavy metal intoxication. The bone age was at the 6 to 7 year level.

Minimal proteinuria, glucosuria and acinoriduria of a non specific type were observed throughout the hospitalization. Additional laboratory data are given in Table 1.

The child's stools contained *Trichuris trichiura* and hookworm ova for which he was treated after completion of the study.

METHODS

The patients were selected according to the following three criteria: treatment for plumbism for the first

time a blood level of at least 70 μg per 100 ml and no necessity for emergency treatment. They were studied in the Yale Children's General Clinical Research Center. After an initial period of 3-6 days during which the child became adjusted to the hospital environment and the diet was prepared there followed 8 three day periods.

I and II No therapy.

III and IV D-penicillamine 100 mg/kg/day in divided doses given every 6 hours orally.

V and VI No therapy.

VII and VIII BAL 24 mg/kg/day in divided doses given every 4 hours and EDTA 75 mg/kg/day mixed with procaine in divided doses every 4 hours given intramuscularly.

Each patient received three different daily menus alternating for each 3 day period. Duplicates of the food and fluids offered to each patient during the first and last period were assayed for lead content as were the unconsumed portions of each meal. The diets for periods I and VIII and the left overs for all the periods were each homogenized and portions were frozen until analysed. The difference between the average of the lead consumed during the first and eighth periods and the lead content of the unconsumed food was taken to represent the lead intake.

All samples were collected and stored in acid washed bottles. Twenty four hour urine samples were collected on each day of the study with the exception of period V. The daily urines were then combined into a single sample for each 3 day study period and an aliquot was frozen until used for analysis.

Stools were collected for 72 hour periods using alternating charcoal and carmine red markers. The collections began with the first appearance of the marker and ended with the first evidence of the next

Patient	I	II	III	IV	VI	VII	VIII
100	99	99	103	94	102	96	1000
36	34		18	15	20		
95	11	133	77	87	116		
51	60	53	46	51	61		
79	21	36	27	31	28		
13	12	7	18	7	6		
		0.5			0.2		
		28			151		
		0			0		
		0.1			0.0		
		2			1		
		11			7		
			150				
							1
							2.0

marker. All stools were homogenized and an aliquot was kept frozen.

Lead determinations on the blood and cerebrospinal fluid were performed by the Laboratory Division of the Connecticut State Department of Health on ashed aliquots by the dithione extraction method as modified by Woessner & Cholak (11) and Cholak et al (7). Urin, stool and diet specimens were examined for lead by the same method at the Occupational Medicine Service Industrial Hygiene Laboratories of the Massachusetts Institute of Technology.

On the last day of each period blood was obtained for a complete blood count for blood lead and for determination of electrolytes, protein, calcium, magnesium, iron, phosphorus and ceruloplasmin concentrations and for liver function tests. Urine was tested for creatinine, protein and glucose excretion and for amino acid and sugar chromatography. Spinal fluid was obtained when blood lead levels were high.

RESULTS

Balance studies

These are shown in Fig 1 together with the corresponding blood lead concentrations. There was a striking decrease in the blood lead levels to normal range after 5 days of either treatment. All of them showed a rebound however on the sixth day after discontinuation of the first medication, D-penicillamine. The increase in lead excretion in the urine during the periods of treatment was striking and there was a direct relationship between the blood lead con-

centration before treatment and the amount of lead excreted. In all patients the stool content of lead was higher than that ingested in the diet with the exception of the first two control periods on Case 1.

The quantities of lead excreted during the treatment periods are shown in Table 2.

Other studies

Serum electrolytes, protein, calcium, phosphorus, iron and magnesium levels as well as ceruloplasmin concentrations remained constant during the 8 periods.

Several abnormalities of the blood were noted (Table 1). There was a mild reticulocytosis in all 3 patients. An eosinophilia was present in all. Neutropenia was noted once in Case 1 during period VI.

Liver function tests on all patients showed an increase of SGOT to abnormal values after BAL + EDTA treatment. In 2 of the 3 patients the cephalin flocculation test became strongly positive at 48 hours during the BAL + EDTA treatment; a positive result was also obtained in Case 3 during D-penicillamine treatment. The alkaline phosphatase in Case 2 which was elevated before treatment showed a decrease to the normal range.

No significant changes were observed in urinary amino acids or in the results of routine urinalysis.

DISCUSSION

D-penicillamine appeared to be as effective a chelating agent as BAL + EDTA in these studies. A more definitive comparison cannot be made on the basis of our data, however, since D-penicillamine was the first drug administered and therefore may be presumed to have interacted with a larger absolute and proportional amount of "labile" lead. More rigorous comparison would require alternation of the sequence of administration of the drugs and greater number of cases. The adequacy of D-penicillamine as a plumburetic agent is established, however, from the present as well as previous studies (1, 6, 10).

LEAD BALANCE STUDIES

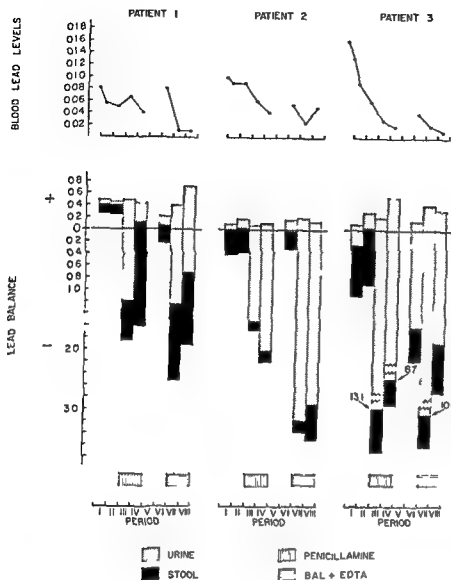


Fig 1 The effects of chelation agents upon lead balance. Blood lead levels are expressed in mg/100 ml. During the indicated periods the top of the bar indicated the amount ingested (mg/period) the urine and stool lead content are then plotted negatively so that the total height of the bar represents the lead excretion in mg/period.

It is pertinent that the administration of D-penicillamine was not especially regulated with respect to food ingestion. It is quite possible that its efficiency would have been greater were it given on an empty stomach as is customary in treating patients with Wilson's disease.

The dosage of D penicillamine which we em-

ployed was higher than that implied by Gibbs & Walshe (4) and by Chisolm (1) to be a safe maximum about 40 mg/kg/day. According to these authors the minimum time preceding the development of signs of pyridoxine deficiency was 2 months of uninterrupted use. It should be stressed however, that a D penicillamine dose level as high as 100 mg/kg/day, was well tolerated in our 3 patients. Jaffe et al (7) found that it took 2 weeks of uninterrupted administration of about the same dose as that administered by Gibbs & Walshe (4) to produce biochemical but not clinical evidence of pyridoxine deficiency.

The rebound in blood lead concentration

Table 2 Cumulative negative lead balance during chelation therapy

	D Penicillamine	BAL + EDTA
Case 1	3.47 mg	4.42 mg
Case 2	3.90 mg	6.92 mg
Case 3	22.45 mg	13.90 mg

noted 6 days after discontinuing treatment is not unusual in the use of chelation therapy in general. Repeated courses of treatment until the rebound concentration is within acceptable limits is a standard procedure.

While D-penicillamine has been given parentally in some studies (10) it is its ability to function effectively when administered orally that makes it the preferable drug for chelating the asymptomatic child. The pain, restraint and bewilderment accompanying injections can be avoided. This is an advantage especially in the management of children of the age and socioeconomic background most likely to have accumulated lead. It must be remembered however that chelators are transporters and that their affinities for their ligands are relative not absolute. "Dietary" lead thus could be transported to the tissues by orally administered D-penicillamine so that the latter should not be used unless it is certain that no lead remains in the intestinal tract and that the child is no longer exposed to the source.

Although potentially serious hematological difficulties have been encountered early in the course of penicillamine treatment (8) these have been reversible and would not appear to contraindicate the use of the drug. In our patients neutropenia was noted only once and this was moderate and transient. The mild reticulocytosis noted in our patients was noted on admission in one and may in the others have been due to blood sampling or to the lead accumulation itself. The eosinophilia noted in 2 patients was present at the time of admission.

It is of interest that the only sign of liver toxicity, an elevation in transaminase, occurred during BAL-EDTA administration and that no urinary abnormalities were noted during either regimen.

We conclude that short term oral administration of D-penicillamine is a convenient and effective means of removing lead from the body provided that (i) the patient is well enough to be cooperative, (ii) has no lead in his gut and (iii) is no longer exposed to inestimable lead containing material. Furthermore, this use of

penicillamine would appear to offer no more and more likely, less of a potential hazard than BAL+EDTA or than EDTA alone. It is to be stressed that both regimens are potentially toxic and require careful monitoring.

SUMMARY

Oral D-penicillamine and intramuscular BAL+EDTA have been used in the treatment of lead accumulation in three children with blood levels of at least 70 μg per 100 ml. Lead balance studies were done during control periods and while the children were given either D-penicillamine orally or BAL plus EDTA intramuscularly. There was a marked cumulative negative lead balance and the blood lead levels decreased to normal after 6 days with either treatment. The results indicate that D-penicillamine given to three children orally in doses as high as 100 mg/kg/day is an effective and non-toxic lead chelator for short term use.

ACKNOWLEDGEMENT

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LEAD BALANCE STUDIES

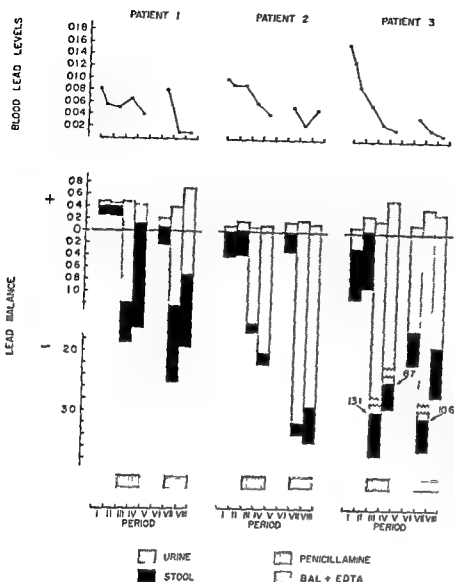


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- Key words** Lead poisoning plumbism penicillamine
BAL EDTA chelators

INTERMITTENT MUSCULAR WEAKNESS EXTRASYSTOLES AND MULTIPLE DEVELOPMENTAL ANOMALIES

A New Syndrome?

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The co-existence of multiple anomalies in a patient has always attracted physicians attention particularly if the patient's appearance deviates grossly from normal. Usually in such a case an attempt is made to find a single pathogenic factor. In a few cases the diagnosis suggests itself to the physician already after his first glance at the patient. Examples of this kind include the syndromes of Marfan and Down. Most cases however are much more difficult to clear up and in a number of instances no definite diagnosis can be established.

In the following paragraphs the case history of an 8 year-old boy will be presented. He has several signs and symptoms suggesting a syndrome. However on searching the literature we have not been able to find a description which fits our case. It is hoped that this paper might prompt other physicians to report on similar patients.

CASE REPORT

The patient was born in June 1962. His mother had previously had four abortions but has given birth to a normal girl in 1966. His father suffers from psoriasis. Otherwise both parents are healthy. His mother's sister has primary amenorrhoea. Apart from that, the family history has revealed nothing of importance.

The pregnancy was complicated by a minor vaginal bleeding in the second month for which the mother was treated with hormone injections of unknown type. The boy was born at term and delivery was normal. His birthweight was 3 000 g. Several developmental anomalies were noticed soon after birth: a large soft cranium with incomplete mineralisation of the frontal and parietal bones; a defect of both the soft and the osseous palate; a single transverse palmar crease of both hands; and cryptorchidism. Three months later extrasystole was found at a local hospital but no other cardiac signs or symptoms were present. Control at irregular intervals during the following years showed an unchanged frequency of extrasystoles. Physical development seemed to proceed normally: the boy was able to sit without support at the age of 7 months and he could walk 7 months later. Since an unsuccessful attempt at surgical repair of the palatal defect in 1964 the boy has been wearing a palatal plate.

In June 1969 the boy now aged 7 was admitted to a local hospital after an episode of muscular weakness of 3 / hours duration during which he had been unable to raise his arms and legs. The most prominent finding was coupled ventricular extrasystoles because of which he was digitalized. He was discharged on a regimen of digoxin 0.125 mg per day. This treatment did not seem to have any effect on the number of extrasystoles.

One month later he was readmitted after a short lasting syncope and a dizzy spell followed by muscular weakness. No cyanosis, dyspnoea, convulsions or nystagmus were noted. On June 15th 1969 the patient was transferred to the Paediatric Department Rigshospitalet Copenhagen for further investigation.

On admission he measured 110 cm which is 18 cm below normal for his age. His bodyweight was 16.1 kg this is 2 kg below normal for his height. His appearance was peculiar with scaphocephalic skull

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A New Syndrome?

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The co-existence of multiple anomalies in a patient has always attracted physicians attention particularly if the patient's appearance deviates grossly from normal. Usually in such a case an attempt is made to find a single pathogenic factor. In a few cases the diagnosis suggests itself to the physician already after his first glance at the patient. Examples of this kind include the syndromes of Marfan and Down. Most cases however are much more difficult to clear up and in a number of instances no definite diagnosis can be established.

In the following paragraphs the case history of an 8 year-old boy will be presented. He has several signs and symptoms suggesting a syndrome. However on searching the literature we have not been able to find a description which fits our case. It is hoped that this paper might prompt other physicians to report on similar patients.

CASE REPORT

The patient was born in June 1962. His mother had previously had four abortions but has given birth to a normal girl in 1966. His father suffers from prostatic hypertrophy. Otherwise both parents are healthy. His mother's sister has primary amenorrhoea. Apart from that, the family history has revealed nothing of im-

The pregnancy was complicated by a minor vaginal bleeding in the second month for which the mother was treated with hormone injections of unknown type. The boy was born at term and delivery was normal. His birthweight was 3 000 g. Several developmental anomalies were noticed soon after birth: a large soft cranium with incomplete mineralisation of the frontal and parietal bones; a defect of both the soft and the osseous palate; a single transverse palmar crease of both hands and cryptorchidism. Three months later extrasystole was found at a local hospital but no other cardiac signs or symptoms were present. Control at irregular intervals during the following years showed an unchanged frequency of extrasystoles. Physical development seemed to proceed normally: the boy was able to sit without support at the age of 7 months and he could walk 7 months later. Since an unsuccessful attempt at surgical repair of the palatal defect in 1964 the boy has been wearing a palatal plate.

In June 1969 the boy now aged 7 was admitted to a local hospital after an episode of muscular weakness of 3 / hours duration during which he had been unable to raise his arms and legs. The most prominent finding was coupled ventricular extrasystoles because of which he was digitalized. He was discharged on a regimen of digoxin 0.125 mg p r day. This treatment did not seem to have any effect on the number of extrasystoles.

One month later he was readmitted after a short lasting syncope and a dizzy spell followed by muscular weakness. No cyanosis, dyspnoea, convulsions or nystagmus were noted. On June 15th 1969 the patient was transferred to the Paediatric Department Rigshospitalet, Copenhagen for further investigation.

On admission he measured 110 cm which is 18 cm below normal for his age. His bodyweight was 16.1 kg, thus 2.1 kg below normal for his height. His appearance was peculiar with scaphocephalic skull



Fig 1 Eight year old boy with low set ears hypertelorism broad root of the nose mandibular hypoplasia and scaphocephalic cranium

hypertelorism low set ears broad nose hypoplasia of the mandible thin hair and slight bilateral ptosis (Fig 1) Body proportions were normal His fingers were short and pointed with an inward bending of the fifth fingers (Fig 2) and his fifth toes were overlaid His testicles could not be palpated No cardiovascular abnormalities could be demonstrated apart from an irregular heart rate resulting from extrasystoles There was no praecordial thrill and no significant murmurs The second sound over the pulmonary area was normal A phonocardiogram was also normal An ordinary neurological examination disclosed weak patellar reflexes on both sides but normal muscular strength and tone There was no sign of dyscoordination and the cutaneous sensibility was normal

An intraoral examination showed normal mucous membranes In the central part of the palate a defect measuring $1 \times \frac{1}{2} \times 2$ cm was found while the posterior part of the hard palate and the soft palate was characterized by cicatricial changes and by lack of uvula (Fig 3)

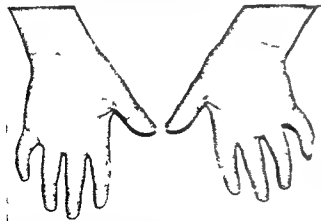


Fig 2 Hands with short and pointed fingers inward bending of the fifth fingers and transverse furrow in the palms

The following teeth were present (nomenclature according to Haderup (3))¹

05	04	03	02	01	+	01	02	03	04	05	
6	05	04	03	02	01	-	01	02	03	04	05

All teeth were of normal shape and colour There was a cross bite on +04 -04 +05 -05 04+ 04- 05+ and 05- and 02+ was inverted lingually There was an open bite in the front region measuring 4 mm Radiological examination showed aplasia of 7- 5- 4- -4 -5 -7 1- and -1 The stage of development of the tooth germs was late

¹ According to the Haderup system of dental designation + signifies the maxilla - the mandible If the symbol is placed to the right of the figure the right side is indicated and vice versa 0 before the figure indicates a primary tooth



Fig 3 Central defect of the hard palate surrounded by cicatricial changes after surgical intervention

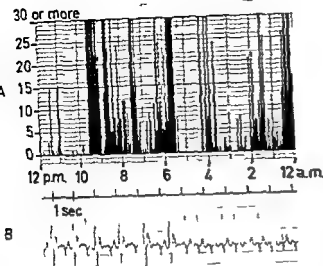


Fig 4 (A) 12 hour histogram showing the number of ectopic beats per minute. Read from right to left. (B) Characteristic rhythm strip demonstrating a change from bigemini to normal sinus rhythm. Bipolar chest lead ectopic beats indicated by dots.

The laboratory tests referred to below have been divided into different groups in order to evaluate the following diagnostic possibilities.

A Chromosomal anomaly

Cultures of both leucocytes from the blood and cells from a skin biopsy showed a chromosomal pattern as found in the normal male.

B Neuromuscular disease

Electromyography of the right biceps and left anterior tibial muscles and of the hypothenar muscles after repetitive stimulation of the ulnaris discloses no signs of neuromuscular disease. The blood concentrations of the following intracellular enzymes were all normal: glutamate pyruvate transaminase (0.5 units/liter), lactic acid dehydrogenase (LDH) (20 u/l) with a normal distribution of LDH isoenzymes, aldolase (2.2 u/l) and alkaline phosphatase (78 u/l). The creatine phosphokinase was slightly but not constantly elevated (141–45 u/l). The urinary excretion of creatin and creatinin was also within the normal range for his age: 50 mg and 390 mg respectively. Serum potassium was 4.7 mEq/l. These investigations were carried out when the patient was free from symptoms.

C Endocrine disease

The plasma level of protein bound iodine (51 µg/100 ml), the uptake of radioactive triiodothyronine by the patient's serum and the urinary excretion of 17 keto-steroids (0.4 mg/day) and 17 ketogenic steroids (3.7 mg/day) were all normal. The administration of metyrapone resulted in an increased excretion of 17 ketogenic steroid in the urine (12.5 mg/day) and a significant fall of the plasma cortisol (1.5 µg/100 ml) while compound S increased (17.7 µg/100 ml). X-ray of the skull (including sella turcica) showed a relative underdevelopment of the facial bones and several ossa Wormiana but was otherwise normal.

X-ray of the whole skeleton showed no abnormalities; the centres of ossification and the epiphyseal lines were normal.

D Other investigations

Haemoglobin 14.2 g/100 ml, Leucocyte count 4300/mm³, Prothrombin time 107%, Serum sodium 146 mEq/l, Serum calcium 10.4 mg/100 ml, Serum phosphorous 3.4 mg/100 ml, Blood urea 14 mg/100 ml, Serum creatinine 0.6 mg/100 ml, Urine microscopy normal, IgA 0.93 g/l, IgG 10.9 g/l, IgM 0.41 g/l, Leucocytes for lysosomal enzymes normal, Urinary excretion of amino acids and acid mucopolysaccharides normal. An electroencephalogram was taken on three different occasions at approximately 6-month intervals, including tracings during photostimulation, hyperventilation and slight dose. Real sleep was not achieved despite adequate sedation. All tracings were normal. Ophthalmological examination normal, Audiometry normal. The vestibular function was not investigated. Intelligence quotient 96 (Binet).

E Cardiac disease

Because of multiple extrasystoles the patient was transferred to Medical Department B, Rigshospitalet, Copenhagen.

Cardiovascular findings were unchanged. Peripheral pulsation was normal. Arterial blood pressure was 110/70 mmHg. The chest roentgenogram showed a normal cardiac silhouette and pulmonary fields.

The ECG was monitored continuously by telemetry during the whole of his 7 weeks stay in hospital. Multiple extrasystoles, probably of ventricular origin, were present most of the time. The percentage of extrasystoles varied from 0–84% usually being in the range of 30–40%. A representative rhythm strip together with a 12-hour histogram is shown in Fig. 4. Occasionally series of 3–5 extrasystoles were noted (Fig. 5). Exercise was not consistently associated with



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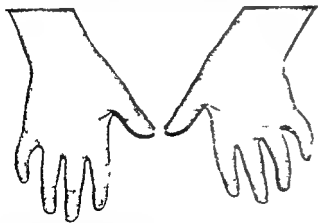


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pulmonary stenosis cardiac conduction disturbances of impulse formation and conduction are present (2). However the physical findings chest roentgenogram and electrocardiogram in our patient did not support the diagnosis of a valvular anomaly. There were no lentiginosities of the skin and the patient's hearing was normal.

In neuromuscular diseases the myocardium is often affected and often disturbances of impulse formation and conduction are present (5). The normal size and shape of the heart in this patient together with an essentially normal ECG (in the absence of extrasystoles) and the absence of cardiac symptoms make the diagnosis of myocardiopathy unlikely.

In certain muscular diseases as for instance the progressive muscular dystrophies the blood concentration of intracellular enzymes such as creatine phosphokinase, aldolase, lactic acid dehydrogenase and glutamate pyruvate transaminase are markedly elevated (10). In our patient the elevation of the serum level of creatine phosphokinase was only slight and intermittent and the levels of all the other enzymes mentioned were normal also during periods of muscular weakness. The same applies to potassium which together with the character of the episodes of muscular weakness speaks against a muscular disease associated with disturbances of potassium metabolism (adynamia episodica hereditaria, familial periodic paralysis (1, 2)). The urinary excretion of creatin and creatinine was normal. Furthermore the electromyographic findings disclosed no abnormalities which also speaks against the possibility of myasthenia gravis which might have been suspected because of the intermittent character of the muscular weakness (6).

The possibility that the symptoms could be caused by an epileptic equivalent such as psychomotor epilepsy does not seem very likely. The EEGs were all normal but unfortunately no curve was obtained during sleep. However the symptoms were by no means characteristic of psychomotor seizures and the patient was fully conscious during the attacks.

Concerning the other characteristics of our patient the reason for his dwarfism remains obscure. Plasma levels of protein bound iodine and cortisol were normal as was urinary excretion of 17 ketosteroids and 17 ketogenic steroids. Pituitary reserve of corticotropin was not decreased as judged by the metyraponetest. Sella turcica was of natural shape and size and skeletal development was not retarded.

The peculiar appearance of the patient's face has a certain resemblance to the syndrome of bird-headed dwarfism (8) but other essential features of this syndrome are missing (especially mental retardation). Further this syndrome does not include any cardiac signs.

The palatal defect, the mandibular hypoplasia and the aplasia of some of the teeth were of no further help in the classification of the patient.

In all the above mentioned syndromes a hereditary factor seems to play an important role. The family history of our patient does not support this possibility. The pregnancy was complicated by vaginal haemorrhage in the second month and the mother was treated with hormones. This may in an unknown way have been of some importance for the pathogenesis of the developmental anomalies in our patient.

It is tempting to suggest that the presence in a child of symptoms and signs involving a number of organs and tissues be due to a chromosomal anomaly. Even if we have not been able to demonstrate one this remains a possible explanation of the findings in our case as current techniques only allow visualization of very gross abnormalities of chromosomal structure involving several thousand genes.

SUMMARY

A description is given of an eight year old boy with extrasystoles, seizures of muscular weakness and multiple developmental anomalies (dwarfism, scaphocephalic skull, hyperlordism, bilateral ptosis, low set ears, broad nose, mandibular hypoplasia, aplasia of a number of teeth, defect of both the soft and os-

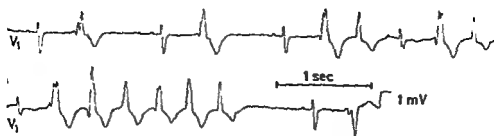


Fig 5 ECG showing sinus rhythm and premature beats (ectopic)

more extrasystoles than were present at rest. A 12 lead ECG was essentially normal but sometimes a slight ST depression and inversion of T waves were present in leads V_1 through V_6 (Fig 6). On the assumption that his syncope and dizzy spells might have been caused by runs of extrasystoles a therapeutic trial was undertaken. The following regimens tried in succession over a period of 7 weeks failed to reduce the number of extrasystoles: practolol 225 mg/day, propranolol 100 mg/day and procainamide 1 g/day. N-propylmalmalumbitartrate (Giulini), a new drug which is not yet commercially available in a dose of 20 mg/day did result in a reduction of the number of extrasystoles of about 75%. However because of lack of experience with this drug it was decided not to continue this therapy on a long term basis.

He had no syncope or dizzy spells during this admission. He experienced four episodes of muscular weakness which were witnessed by the staff. On one occasion he was not able to raise his arms on another while walking around in the ward he suddenly fell to the floor. He was not able to rise without help. He could walk but limped. A considerable decrease of muscular strength in the right leg was clearly demonstrated when he tried to climb stairs. He was fully conscious during these episodes.

During such periods of muscular weakness which were not related to any changes in the ECG, serum LDH (including iso enzymes), serum aldolase, serum creatinine phosphokinase and serum potassium were normal. The attacks would last for a few hours. He had had these attacks since early childhood at intervals varying between 1-2 weeks to 1-2 months each episode lasting 1/2 hour to 2 days. There was no constant relation to rest, physical activity or meals. To investigate the latter possibility further a glucose tolerance test including determinations of serum potassium was performed and was found to be normal.

The patient was discharged September 1970 and has since then been seen in January and in July 1970 in Pediatric Department G. In this period the seizures of muscular weakness have decreased. His ECG was recorded by tape-recorder for 2 1/2 hours in July 1970. On average the number of extrasystoles was 1 per hour but for shorter periods of time more serious extrasystoles were noted. The child was still free of cardiac symptoms and had no syncope. Physical findings were unchanged.

DISCUSSION

The most outstanding features of the case are extrasystoles, seizures of muscular weakness and multiple developmental anomalies in connection with a characteristic perinatal course. These findings naturally lead us to consider the presence of a syndrome. However we have not been able to find a similar combination of symptoms in the literature.

In a number of syndromes cardiac and symptoms are prominent (9). Holt-Oram syndrome is characterized by a combination of disturbances of cardiac rhythm, atrial septal defect and skeletal defects of the upper extremities (5). In our patient there was no evidence to suggest the presence of a septal defect. The roentgenogram disclosed no malformation of the skeleton.

In Gorlin's Leopard Syndrome (10) the

pulmonary stenosis cardiac conduction disturbances of impulse formation and conduction are present (2). However the physical findings chest roentgenogram and electrocardiogram in our patient did not support the diagnosis of a valvular anomaly. There were no lentiginosities of the skin and the patient's hearing was normal.

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It is tempting to suggest that the presence in a child of symptoms and signs involving a number of organs and tissues be due to a chromosomal anomaly. Even if we have not been able to demonstrate one this remains a possible explanation of the findings in our case as current techniques only allow visualization of very gross abnormalities of chromosomal structure involving several thousand genes.

SUMMARY

A description is given of an eight-year-old boy with extrasystoles seizures of muscular weakness and multiple developmental anomalies (dwarfism scaphocephalic skull hyper telorism bilateral ptosis low set ears broad nose mandibular hypoplasia aplasia of a number of teeth defect of both the soft and os

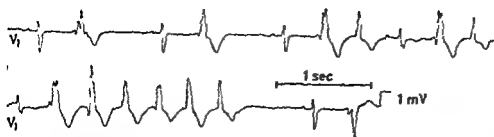


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The patient was discharged September 27th 1969 and has since then been seen in January and again in July 1970 in Paediatric Department G. During this period the seizures of muscular weakness seem to have decreased. His ECG was recorded by a portable tape recorder for 24 hours in July 1970. On the average the number of extrasystoles was unchanged but for shorter periods of time more series of 2-6 extrasystoles were noted. The child was still free of cardiac symptoms and had no syncope. Physical findings were unchanged.

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In Gorlin's Leopard Syndrome, lentigo

Fig 6 12 lead ECG at rest
Paper speed 50 mm/sec

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seous palate, inward bending of the fifth fingers, single transverse palmar crease of both hands, and cryptorchidism) These findings suggest a specific syndrome, but no similar description was found in the literature The investigations disclosed no signs of either a chromosomal, a neuromuscular, or an endocrine disease

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CARBIMAZOLE TREATMENT IN EARLY PREGNANCY

Ultrastructural and Biochemical Observations on the Thyroid Glands of two Twin Fetuses

P OLIN and H EKHOLM

From the Department of Endocrinology and Metabolism Karolinska sjukhuset Stockholm and the Department of Anatomy University of Goteborg Sweden

Thyrostatic drugs are known to pass through the placenta in the human and in animals at least in late pregnancy (1-3 5 7 8). The effect of these drugs on the human fetus in the second trimester of pregnancy is little known although the onset of thyroid function occurs at the conceptual age of 10 to 11 weeks corresponding to a fetal crown-rump length (CR) of 60-65 mm (16 17). The treatment of choice in the pregnant women with thyrotoxicosis is therefore still a matter of debate (1-2 10 12 14). Accordingly it may be pertinent to present the findings in two twin fetuses on the rare occasion when a thyrotoxic woman on carbimazole was subjected to a therapeutic abortion in the 14-17th week of gestation.

CASE REPORT

A 44 year-old married woman was subjected to a therapeutic abortion because of hyperthyroidism and weakness. She became thyrotoxic at the age of 26 years and underwent then a subtotal thyroidectomy with clinical remission postoperatively. She bore 4 normal children the last one born in 1961. In February 1969 she saw her gynecologist because of possible pregnancy her last menstrual bleeding occurring on November III 1968. At the same time she complained of heart trouble. The clinical examination disclosed a blood pressure of 200/120 mm Hg and a cardiac arrhythmia. The thyroid gland was enlarged. There was no exophthalmos but a staring expression. She had fin er tremor. The clinical diagnosis of thyrotoxicosis was verified by a PBI of 14.1 $\mu\text{g}/100\text{ ml}$ (normal value during pregnancy 7-12 $\mu\text{g}/100\text{ ml}$) and a T resin uptake of 119% (normal

range 80-120%). LATS was not detectable by the McKenzie bio-assay. An X ray of the chest was normal. The ECG showed atrial fibrillation. She was treated with carbimazole 15 mg three times daily for 14 days before the operation. During the treatment the goitre decreased in size. The PBI was 7.7 $\mu\text{g}/100\text{ ml}$ and the T resin uptake was 99% at the time of the abdominal hysterotomy on March 24 1969. The twin fetuses of a CR of 120 and 105 mm and a weight of 105 and 81 g respectively showed no external abnormalities. The thyroid glands were not conspicuously enlarged but at the excision the glands appeared more vascular and softer than those of other fetuses of the same size.

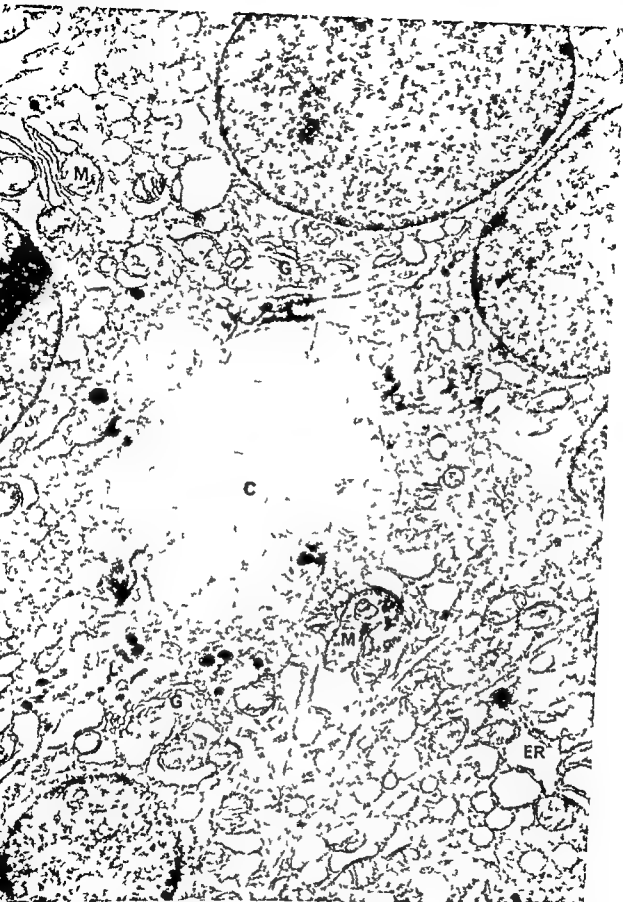
METHODS

The fetal thyroid glands were excised 1 hour after the operation. One lobe from each gland was immediately immersed in a glutaraldehyde fixative and processed as described previously for light and electron microscopy (16). The second lobe was used for biochemical studies as described in detail in a previous paper (17). These lobes were cut in 4-5 pieces and incubated for 4 hours in 37°C in 2 ml of Eagle medium without leucine containing 100 μCi of ^3H leucine (spec act 159 Ci/mM) and 23 μCi of carrier free $\text{Na } ^{125}\text{I}$. The tissues were weighed and the ^{125}I radioactivity was measured in the tissue and the medium. The ratio of the ^{125}I radioactivity per mg tissue/ ^{125}I radioactivity per μl medium was calculated. The soluble proteins were extracted with a phosphate buffer separated by means of linear sucrose density gradient centrifugation and analysed for ^{32}P and ^{125}I radioactivities as described earlier (17).

RESULTS

Morphology

The description given below is not meant as a complete account of the morphology of the



Survey electron micrograph of a thyroid follicle from the smaller twin fetus (CR 105 mm). The lumen is filled with colloid (C) into which villi project from the apical cell surfaces. The

follicle cells have well developed rough surfaced endoplasmic reticulum (ER) and Golgi apparatus (G). Mitochondria (M) and lysosomes (L) are also seen $\times 8000$



Fig 2 Electron micrograph of the apical portions of two follicle cells (fetus CR 105 mm). Microvilli are seen projecting into the dense colloid (C). The cisternae of the rough surfaced endoplasmic reticulum (ER) contain a foculent material. In the cytoplasm a large number of small vesicles are seen as well as a few mitochondria (M) and lysosomes (L). 14 000

culum (ER) contain a foculent material. In the cytoplasm a large number of small vesicles are seen as well as a few mitochondria (M) and lysosomes (L). 14 000

two fetal thyroids only those cell components that are of special significance in judging the effect of an enhanced TSH stimulation are dealt with. The valuation of the observations is based on the results obtained in a previous study on the thyroid of normal fetuses (16).

The light microscopic examination of the fetal thyroids showed that the epithelial cells were organized into groups separated by spaces containing connective tissue and capillaries. Many of these groups appeared compact but in approximately 50% of the groups the cells were arranged concentrically around a lumen which was filled with a substance deeply stained by toluidine blue. These microfollicles were considerably smaller than those of the adult human thyroid and had a proportionally smaller lumen.

Electron microscopical survey pictures (Fig

1) revealed that the microfollicles were composed of cuboidal to columnar cells surrounding a lumen containing a homogeneous substance of high density. The lumina were either clover leaf shaped or rounded. The cell surfaces bounding the follicle lumina were provided with numerous long and slender microvilli (Fig. 2). In addition the apical region of some cells had rounded lumina which were bounded by a membrane projecting into the lumen in numerous microvilli and which contained a homogeneous dense substance. Whether these intracellular lumina are true intracellular cavities or canaliculi extending from the follicle lumen has not been settled in the present study. (For further discussion the reader is referred to Olin, Ekholm & Almquist 1970 (16)). No pseudopodia indicating phagocytosis of colloid were seen on the apical cell surfaces.

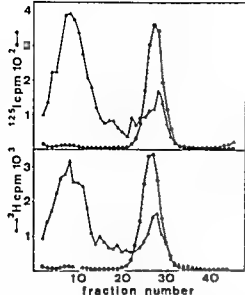


Fig 3 Sucrose density gradient centrifugation patterns of the soluble thyroid extracts from the twin fetuses after 4 hr *in vitro* labelling of the thyroid glands with ^{125}I - and ^3H -leucine. Top: fetus CR 105 mm and bottom: fetus CR 120 mm. The main radioiodine peaks represent thyroglobulin (17). Sedimentation is from left to right. The gradient contains 5–20% sucrose in 0.02 M sodium phosphate and 0.1 M KCl, pH 7.4. Centrifugation for 220 min at 200 000 g. Each fraction is plotted.

The follicle cells (Fig 2) had a well developed rough surfaced endoplasmic reticulum; the basal and middle cell regions were almost filled with large, rounded or elongated cisternae studded with ribosomes on their outer surfaces and containing a flocculent material of moderate density; the apical cell zone contained numerous similar but smaller cisternae. Free ribosomes, generally gathered in clusters, were numerous. The Golgi apparatus was well developed and was characterized by a large number of small vesicles. Similar smooth surfaced vesicles with a content of low to moderate density were also found in considerable numbers in the apical cell zone. The same zone contained many round or oval, very dense bodies interpreted as lysosomes. Very rarely structures were observed in the apical and middle cell zones that could be interpreted as colloid droplets; they were rounded, membrane-bounded bodies, generally somewhat larger than the biggest lysosomes, and containing a

homogeneous substance of high to moderate density.

Biochemistry

The weight of one thyroid lobe in the twin fetuses was 28.5 mg and 25.2 mg respectively (normal range for total thyroid weight 20–70 mg at CR 120 mm (16, 18)). When the lobes were incubated for 2 hours in the presence of ^{125}I - and ^3H -leucine they accumulated ^{125}I - from the medium. The ^{125}I tissue over medium ratio was 19.1 and 24.4 respectively. 88–92% of the ^{125}I radioactivity (total $3.5\text{--}5.0 \times 10^4$ cpm) could be extracted. After extensive dialysis 2.7–7.4% of the ^{125}I radioactivity was left in the dialysis bag and considered to be proteinbound.

Separation of the soluble proteins by ultracentrifugation on sucrose density gradients resulted in a radioactivity peak for both ^3H and ^{125}I in the thyroglobulin region. ^3H labeling was also found in slow sedimenting proteins (Fig 3).

DISCUSSION

There are only two previous reports on the effects of thyrostatic agents on the preterm human fetal thyroid gland. Davis & Forbes (4) reported histological signs of hyperactivity in the thyroid gland of a 24 week old fetus of a thyrotoxic woman treated with propylthiouracil. Freiesleben & Kjerulf Jensen (7) mentioned an increased thyroid weight in a fetus of a crown-heel length of 23 cm, fetal age 19 weeks, whose mother was treated with propylthiouracil for thyrotoxicosis. The present case report of two twin fetuses of a CR of 105 and 120 mm, fetal age 14–17 weeks, is the first presentation of ultrastructural and biochemical studies in human fetuses subjected to thyrostatic drugs given to the mother. In contrast to the earlier reports, the present investigation showed a normal thyroid weight with respect to fetal size, and the light and electron microscopic morphology of the thyroid was normal as compared with our observations on normal

fetuses of corresponding age (16). Thus there were no morphological features indicating a pathologically augmented TSH-stimulation. Pseudopodia on the apical cell surface and numerous colloid droplets indicative of an increased hormone release were not observed. Dilatation of the cisternae of the endoplasmic reticulum and hyperplasia of the Golgi apparatus alterations which are considered to reflect an increased protein synthesis (11, 18) could not be established. The biochemical *in vitro* studies showed a considerable incorporation of ^{125}I into thyroglobulin indicating that the maternal treatment with carbimazole did not cause prolonged inhibition of the organification of iodine in the fetal thyroid gland.

The normal weight, morphology and incorporative capacity of iodine could possibly be due to the relatively short duration of the carbimazole treatment of the mother. However this interpretation does not seem very likely since the treatment was efficient as documented by the reduction of her PBI from 14.1 to 7.7 $\mu\text{g}/100\text{ ml}$ during the 14 day period. There are two other more plausible explanations of the lack of effect of the carbimazole treatment in our study. One concerns the efficiency of the fetal pituitary thyroid feed back system and the other the permeability of the placenta for thyrostatic drugs at this stage of fetal development.

In a previous paper we showed that the TSH content of the human fetal pituitary starts to increase at a CR of 60 mm (15). Greenberg et al (9) found that the TSH and free thyroxine concentrations in the fetal plasma are rising from approximately the same stage of development. These findings demonstrate that TSH is secreted and indicate that the thyroid is sensitive to TSH. On the other hand they do not answer the question whether the fetal pituitary at this stage of development is sensitive to changes in the plasma level of free thyroxine. Indeed the data of Fisher et al (6) suggest that the fetal thyroid pituitary feed back system is not functioning until the 18th to 22nd week of gestation. Thus the lack of ef-

fect of carbimazole in our fetuses in the 14-17th week of gestation may be explained by an immature feed back system.

It is well established both in animals and in man that thyrostatic drugs pass through the placenta at term (1-3, 5, 7, 8, 13) but nothing is known about this passage in the second trimester. As we know from a previous study (15) that propylthiouracil inhibits iodine organification in the human fetal thyroid even from a CR of 65 mm it cannot be excluded that the absence of visible carbimazole effects in the present study at least partly is due to a restricted placental permeability for this drug.

Our findings of normal structure and normal capacity of iodine incorporation *in vitro* in the fetal thyroid in the 14-17th week of gestation after a short treatment of the mother with high doses of carbimazole do not contravene the suggestion of Herbst & Selenkow (12) that thyrotoxicosis during pregnancy can be treated with small doses of thyrostatic agents. However the maternal fetal and thyroid pituitary relationships at different stages of development are far from unravelled. Further experimental and clinical studies are necessary before the optimal treatment of thyrotoxicosis during pregnancy should be formulated.

SUMMARY

This paper presents ultrastructural and biochemical studies on the thyroid glands from two twin fetuses of a CR of 105 and 120 mm whose thyrotoxic mother was treated for 14 days with carbimazole. The light and electron microscopic appearance of the fetal thyroids corresponded to that normally seen at this stage of development. No morphological signs of excessive TSH stimulation were detected. The fetal thyroid glands incorporated radioiodine and labelled leucine into thyroglobulin *in vitro*. These normal findings during thyrostatic treatment of the mother are discussed and related to the efficiency of the fetal pituitary thyroid feed back system and to the passage of thyrostatic agents through the placenta.

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SHORT TERM DIFFERENCES OF INFANT DEVELOPMENT IN NURSERY HOMES AND IN PRIVATE FAMILIES

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Medical practice has changed in two major respects as a result of the focus on maternal deprivation. The dramatic research results from several countries published by R. A. Spitz (11, 12) in the forties and the extensive overview provided by John Bowlby (1) for the World Health Organization contributed to a sharp decline in the willingness to separate mother and child during the first years of life. Secondly, they sparked efforts towards creating more personalized, stimulating and emotionally warm wards for those children who were being deprived of their parents' care.

The key term, maternal deprivation, has remained controversial, however.

In the psychologically unhealthy institutions studied by Spitz, Dennis and Narayan and others, all of the important sensory modalities were understimulated. It may be appropriate therefore to refer to the disturbances found among institutionalized children (at least in those cases where separation from the mother occurred before the age of 6 months) as perceptual deprivation, including tactile and kinesthetic, rather than using the too-broad and yet too-specific term, maternal deprivation (3).

In his first research report concerning this problem, Spitz stated:

Two factors, both already stressed by Durfee and Wolf, are made responsible by most of the authors for the psychological injury suffered by these children:

First, lack of stimulation. The worst offenders were the best equipped and most hygienic institutions which succeeded in sterilizing the surroundings of the child from germs but which at the same time sterilized the child's psyche. Even the most destitute of homes offers more mental stimulation than the usual hospital ward.

Second, the presence or absence of the child's mother. Stimulation by the mother will always be more intense than even that of the best trained nursery personnel. Those institutions in which the mothers were present had better results than those where only trained child nurses were employed. The presence of the mothers could compensate for numerous other shortcomings (11).

While Spitz concluded that the second factor was the most consequential, no other research reports have been found by this author that provide solid empirical proof for that conclusion. Its widespread acceptance is based on theoretic considerations as much as on empirical information. The psychoanalytic thesis that perception is a function of libidinal cathexis is certainly necessitated, assigning more weight to maternal than to perceptual deprivation during the first year of life (5).

The nature of this conflict is such that experimental efforts to provide conclusive empirical evidence would be ethically unacceptable. Answers to secondary questions raised by the central conflict can be found, however. The present investigation is an attempt to measure short-term developmental effects of deprivation of continuous and emotionally intense parental care. An experimental group of 22 institutionalized infants was given the Hetzer-Bühler baby tests at 6 and 8 months of age and compared to a control group of infants in private families. Attempt was made to control for quantity and in some respects for quality of parental care. Such measurement was possible in Oslo, Norway, where a number of small

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were placed in nursery homes because their mothers were unwell had to work and could not manage to take care of their child. Four other babies were like *w* were born to unwell mothers but in these cases the public Child Welfare Agency had decided the mother unfit to be responsible for her child (These were children *l*, *e*, *e* and *m*). Reasons for placement of the remaining eight babies could not be obtained from official records. This implies the reason could not be the same as for the four previously mentioned and communication from nurses involved in their care suggested these babies were placed by unwell mothers who materially were unable to take care of them.

The second sample was composed of babies living with their own mothers. These subjects were selected at a public Well Baby Clinic in Oslo. Only two of these mothers were employed and both had adequate baby sitting facilities.

The same four criteria were followed in the selection of both samples: health, age, sex and social class background. In both groups the first 22 children to satisfy the criteria were selected for testing. The health criterion involved that the baby should not have suffered any prolonged or serious disease or have been hospitalized for periods exceeding one week total. Neither should he appear retarded in any way or suffer impairment of any sense organ. Child *b* in the nursery sample was a borderline case in this respect. He was hard to communicate with at six months and it was suspected that his hearing might be impaired. Medical examinations were negative however and by 8 months he was alert and outgoing.

The babies were all to be 6 months at the time of the first testing. Computation of average age for family children gave $\bar{x}=6.00$ with $s=7$ and for nursery children $\bar{x}=6.02$ with $s=12$. At second testing the ages were $\bar{x}=7.28$ with $s=8$ for family children and $\bar{x}=8.01$ with $s=12$ for nursery children. The sex distribution was 10 girls to 12 boys in the private families and 11 to 11 in the nursery homes.

It was impossible to obtain a measure for social class background which would include all the babies in the nursery homes. The official records were entirely lacking in information of occupational or educational status and the investigator had to rely on information given by nurses and administrators who had contact at some point with the mothers of these babies. The estimates were consistently given as predominantly working class, some lower middle class. According to this standard the children from private families were selected 8 from social stratum V, 11 from stratum IV and 3 from stratum III. Assignment to social stratum was done on the basis of father's occupation in correspondence with the five point scale developed by Svalastoga in his major work on social stratification in Scandinavia (13).

The reality of the assignment to social class was illustrated by the variety of life situations incidentally noted within the sample. Child *F* was living with her unwell mother in grandparents' apartment L and *w* with their teen age mothers mostly in

grandparents' apartment with father largely absent and *O* lived with her mother and her mother's daughter of an earlier marriage, the husband being recently evicted by court order for alcoholism.

REARING PRACTICES

Quantitative aspects. Most of the mothers in the private homes had more than one child and none had outside help in the house. The ratio of nurses to children in the nurseries was better than 1 to 3 and the amount of work other than caring for the children considerably less than in a home. Each nurse had a couple of children as hers so that each child normally was handled by a small number of nurses and not rotated among the whole staff. It is therefore not necessarily true that an average child would spend more time in direct contact with his parents than a child in a nursery home would spend with two particular nurses.

With respect to specifics of daily routine the nurseries and private families compared as follows for children 7 months of age. Number of hours awake per day varied from 6 to 12 in private homes with 8 and 9 hours being most common. The nurseries were more uniform with most children awake approximately 8 hours daily. All children were fed four times a day except three of the family children who had only three meals. Each meal was estimated to take 10 to 15 min by the mothers; this was also the approximate time for a meal in the nurseries. Again the variation was somewhat greater in the families: one mother estimated 5 to 30 min and one 30 to 60 min. No meal in the nurseries was observed to last longer than 20 min.

Meal times were said to be regulated to some extent by the children themselves in all families except one. Probably the extent to which this happened was smaller in the nurseries. The meals there occupied a full hour and within that time period there was no set order. A child showing clear signs of hunger would be fed first.

Diapers were changed four times daily in all families except four who had five changes and two who had six. Diapers were changed in connection with every meal in the nurseries making four changes the basic norm. Occasionally a child would be changed if he showed discomfort between meals or he would be changed if he was so wet as to be unpleasant to handle.

Ten of the mothers said they usually picked their baby up if he was crying. Four of these added "if he is not crying to be picked up". Four mothers gave a clear no to the question "two said, after a while and one said only if in pain". Fear of spoiling the baby was given as reason for other than affirmative answer. The same attitude was expressed by several nurses. The situation was different in the nurseries however because of their more clearly defined wake and sleeping hours. The investigator often heard babies cry during sleeping hours. In about 50% of these instances a nurse

nursery homes have been changing their organization, staffing, and child caring procedures through the fifties and sixties towards a closer approximation of family type care in a varied and stimulating environment. They do, however, maintain the following characteristic which makes them inadequate with respect to continuous parental care. Those close relationships that are established are regularly broken because virtually all the nurses are doing their internships and never stay longer than one year in one institution.

It was decided to test the infants at 6 and 8 months of age so as to obtain a measure of development during that period. Texts in child psychology (8) generally list 6 months as the age when an infant normally starts differentiating between individuals. John Bowlby (2) states that infants who lose their mothers after this point fret, while those who lose them earlier do not. Therefore, the 2 months after 6 months of age were expected to be particularly sensitive to discontinuous and less emotionally intense parental care.

METHOD

The private families were visited twice. The first visit lasted for little more than one half hour and was occupied with testing the 6 months old infant. The second visit lasted close to one hour and included an informal interview with the mother. Some notes were taken during the talk and the following questions were always included although in varying order and with follow up questions to clarify specific points: How many times a day is your baby fed? How long does the feeding take? How are the feeding times determined—does the child influence them? How often (day average) do you change his diapers? Is the baby picked up when he is crying? How often do you pick him up for other reasons? How long is he awake each day? Where does he spend the time he is awake? Are there people in the same room with him? Who? How is he placed when awake? Is he able to see what is happening in the room? How many and what toys does he have?

The nursery homes provided opportunity for direct observation of childcaring practices. Prior to testing the investigator spent hours most days of the week for 2 months in nursery home I. Ostensibly he was practicing and learning how to use his tests. This

provided the advantage normally associated with participant observation of becoming an accepted part of the milieu and being able to observe its normal functioning. Notes were taken during this time immediately after leaving the nursery home.

Particular interest focused on the character of the relationships between nurse and child in feeding and other situations involving handling. Easily objectifiable observations such as number of feedings were checked with the nurses for accuracy.

The basis for description of the other nursery homes was not equally thorough. Nurseries III and IV were visited ten times each and found virtually identical to nursery I with respect to the characteristics described in this report. Nurseries II and V were visited only three times each. While this was insufficient for making any positive statement as to identity of procedure, no marked difference in character was noted by the investigator. Nursery II differed in being slightly disorganized due to shift of chief administrator at the time of the investigation.

The children were distributed as follows: Nursery I a b c d e f g h i j k l; Nursery II m n; Nursery III o p q; Nursery IV r s t u; and Nursery V v. Only three subjects were therefore placed in insufficiently characterized environments. The great similarity in character of the nursery homes was further corroborated by the circumstance that they all were responsible to the same government agency for the care of the children. The administrators and pediatricians at the different homes maintained a high level of professional communication.

The infants were given the Hetzer-Buhler developmental tests (7) at ages 6 and 8 months. Although these tests are not standardized for Scandinavian countries, they are the ones most widely used and they have been shown to differentiate well between children from different environments (9, 10).

The procedure of testing and the computation of results followed closely that outlined in the English edition of 1935 with three minor exceptions. Item 2 series VI, item 7 series VI, and item 5 series IX were omitted because of technical difficulties. In tabulation of the results these three items were counted as passed if one or more other items in the same series was passed.

For the family children the tests were administered in the home at an hour when the infant was awake and active. The nursery children were given the tests in or around their beds at wake hours. A happy and interested atmosphere was secured for every test period.

SUBJECTS

Two samples each including 22 infants were selected for the investigation. One group came from five different nursery homes in the Oslo area. Average age upon placement was 3 weeks and all subjects had been in the same nursery home from the time they were first placed there. Ten of these babies

were placed in nursery homes because their mothers were unwell had to work and could not manage to take care of their child. Four other babies were like *w* born to unwell mothers but in these cases the public Child Welfare Agency had decided the mother unfit to be responsible for her child (These were children *b c e* and *m*). Reasons for placement of the remaining eight babies could not be obtained from official records. This implies the reason could not be the same as for the four previously mentioned and communication from nurses involved in their care suggested these babies were placed by unwell mothers who materially were unable to take care of them.

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Meal times were said to be regulated to some extent by the children themselves in all families except one. Probably the extent to which this happened was smaller in the nurseries. The meals there occupied a full hour and within that time period there was no set order. A child showing clear signs of hunger would be fed first.

Diapers were changed four times daily in all families except four who had five changes and two who had six. Diapers were changed in connection with every meal in the nurseries making four changes the basic norm. Occasionally a child would be changed if he showed discomfort between meals or he would be changed if he was so wet as to be unpleasant to handle.

Ten of the mothers said they usually picked their baby up if he was crying. Four of these added "if he is not crying to be picked up". Four mothers gave a clear no to the question "two said after a while and one said only if in pain". Fear of "spoiling" the baby was given as reason for other than affirmative answer. The same attitude was expressed by several nurses. The situation was different in the nurseries however because of their more clearly defined wake and sleeping hours. The investigator often heard babies cry during sleeping hours. In about 50% of these instances a nurse

Table 1 *Developmental Quotients*

	Family children				Nursery children		
	DQ at 6 months	DQ at 8 months	Change in DQ		DQ at 6 months	DQ at 8 months	Change in DQ
A	112	116	4	a	112	113	1
B	136	143	7	b	125	120	-5
C	127	119	-8	c	123	123	0
D	130	130	0	d	122	118	-4
E	132	142	10	e	115	107	-8
F	132	149	17	f	141	129	-12
G	121	131	10	g	111	107	-4
H	130	125	-5	h	117	129	12
I	124	126	2	i	130	117	-13
J	108	122	14	j	138	112	-26
K	121	123	2	k	115	100	-15
L	135	147	12	l	121	114	-7
M	138	139	1	m	120	108	-12
N	125	124	-1	n	122	122	0
O	138	134	-4	o	133	144	11
P	124	136	12	p	118	120	2
Q	127	117	-10	q	136	145	9
R	129	124	-5	r	115	104	-11
S	127	127	0	s	122	124	2
T	117	124	7	t	121	117	-4
U	121	133	12	u	149	145	-4
V	114	120	6	v	126	110	-16
	2 768	2 851	83		2 732	2 628	-104
\bar{x}	125.8	129.6	3.8	\bar{x}	124.2	119.5	-4.7
s	8.2	9.8	7.5	s	9.9	12.7	9.3

All calculations from these values done with 42 degrees of freedom

would come in and lift the baby up change him or put him in a reclining infant seat for a while. This however was true only for babies 5 months or older. The younger infants were normally left to cry by themselves.

During wake hours all children in both samples had one or more attentive adults in the same room with them. Normally the babies were on the floor sometimes in a chair or an infant seat placed in their bed. Six family children were reported to spend much time in a baby carriage. A similarly small number of the nursery children would spend greater portions of their wake hours reclined in beds or carriages. Except in these instances all babies were able to look around at what was happening in the room.

During their seventh month the nursery children would spend one or two hours daily moving around each other on a rug. No such experience was available to the family children. All children had a number of adequate toys to manipulate.

Qualitative aspects. The following observations were done with age group 6 to 8 months. No systematic observation was done with younger children. The ratio of nurses to babies was smaller for the younger ones and the care they received was therefore probably less personal.

With no exceptions the nursing staffs expressed in their work a great fondness for the infants. Very few instances of impersonal or mechanical treatment were observed. The nurses were most often talking to the infants when diapers were changed and the feeding situation involved good contact with the child regulating rate of intake and in many instances following the spoon or cup with his hands. When babies were carried around or held on the lap the situation was normally characterized by delight in simple communication such as giving and taking toys, exchanging smiles and babytalk.

These observations could not be made comparatively. There were however two important differences between relationships of mother and child and those of nurse and child. The first concerns the intensity of love feelings on the part of the mother figure. When nurses finished their employment after one year they expressed sadness to leave their children but their sadness was of course in no way comparable to that of a mother taking final leave of her child.

The children who lost their nurses were observed to be less active and communicative and to eat less. Often they were visibly unhappy for weeks. This constituted the second main difference between living in a nursery and in a home: loss and replacement of

the main parental figure was a regular fact of life in the nurseries

RESULTS

The measured developmental quotients are shown in Table 1. At 6 months a two-tailed *t* test gave probability greater than 0.5 that the two groups belonged to the same population with respect to over all level of development. At 8 months of age the family children scored very much higher than the nursery children and the two-tailed *t* test gave a probability of less than 0.01 that the two groups still could be seen as belonging to the same statistical population. When a two-tailed *t* test was applied to the change in development the probability was shown to be smaller than 0.002 that the two groups belonged to the same population.

The Hetzer-Buhler tests measure development in six different areas and thus allows one to construct developmental profiles to show relative development of different abilities. Fig. 1 shows average developmental profiles for the two groups at ages 6 and 8 months. The family children are shown to be approximately 2 weeks ahead in manipulation at 6 months of age, the other areas tested being equally well developed in the two groups. By 8 months of age the family children are almost 5 weeks ahead in manipulation, 4 weeks advanced in mental production and 2 weeks in bodily control, social reactions and learning.

Some investigators have suggested that retardation in development of institutionalized infants may be due solely to lack of opportunity for specific physical exercises (4). If such was the case one would expect to be able to locate the relatively bad test results of the nursery children to a set of test items—mainly in the area of bodily control—with which they performed very badly. In the present investigation there is some tendency to clustering of bad results in the area of manipulation but none in bodily control. When the individual test items were compared one by one the better results of the family children

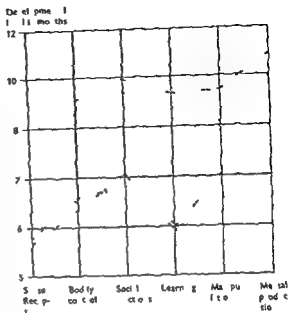


Fig. 1. Average developmental profiles at 6 and 8 months. --- family children, — nursery children.

at age 8 months were spread rather evenly over most items.

DISCUSSION

The sex distribution was slightly uneven in the two groups. The family children had one boy more and one girl less than the nursery children. Average change in DQ among the family children was +21 for boys and +62 for girls. In the group of nursery children the average change was -57 for boys and -47 for girls. Since the girls had a more favorable development in both groups the uneven sex distribution should have diminished rather than increased the difference in development between the two samples.

The information obtained about parental background does not suggest as likely any genetic advantage on the part of the nursery sample. The remarkably similar scores of the two groups at age 6 months therefore suggest that they may have developed at similar rates up to that point.

Explanation of the difference in development

Table 1 *Developmental Quotients*

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The children who lost their nurses were observed to be less active and communicative and to eat less. Often they were visibly unhappy for weeks. This constituted the second main difference between living in a nursery and in a home: loss and replacement of

situation in the nurseries as compared to the private families. The emotional bonds between infant and parental figure appeared less intense and there was a lack of continuity of the relationship.

At 6 months the probability was greater than 0.5 that the two groups belonged to the same population. The nursery group showed a considerable relative retardation in the 2 months thereafter. It is suggested that the 6 first months of an infant's stay in a nursery be sufficient for arrangement of adoption or sufficient improvement in parent(s) life situation for them to take care of the child.

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can hardly be based on an assumption of a generally accelerated development—genetically based—of the family children and a genetically based decelerated development of the nursery children. Since much work has shown such acceleration and deceleration to be expressed considerably earlier than 6 months (6), one should also have to assume a considerably higher—genetically or prenatally based—starting position for the nursery children. This is highly unlikely on the basis of the data presented.

It is noteworthy that institutions which consciously attempt to maintain a personalized and warm atmosphere may succeed equally well as a family in bringing children up to 6 months of age with respect to the many aspects of development measured by the Hetzer-Buhler scales. This differs from earlier authoritative reports on early effects of institutionalization (6). Gesell & Amatruda reported 'diminished interest and reactivity and reduced integration of total behavior' before 12 weeks of age. Before 16 weeks they listed 'beginning of retardation evidenced by disparity between exploitation in supine and in sitting positions'. This should all show up in a Hetzer-Buhler test which is very similar to the Gesell test for ages under one year.

It is equally noteworthy, however, that this general warmth of the environment was not able to maintain an equally favourable development past six months of age. The average developmental quotient was actually lower at 8 months than it was at 6 for the nurseries while a marked improvement had taken place in the families.

Aside from the virtual absence of observed differences in amount and variation of tactile, kinesthetic, and visual experiences, two specific aspects of the results strongly suggest that the significantly more favorable development of the family children was due to characteristics of the relationships between infants and parental figure rather than to understimulation of sensory modalities. When test results were broken down item by item it turned out that

the retardation of the nursery children was spread evenly over all items and all developmental categories. Sensory deprivation in particular might have been expected to show up in the form of inadequate performance on a smaller number of item tests. Secondly, Table I shows that the variability of scores increased dramatically (75%) for the nursery children and much less (27%) for family children over the two month interval when *s* is used as a measure. Such dramatic increase in variability could be a result of the discontinuous and less emotionally intense parental care which affected the nursery children at different times and to various degrees. Possible sensory poverty of the environment should influence all nursery children more uniformly.

Two policy recommendations follow from these findings. Nursery homes should terminate their care for infants by the time they reach 6 months of age. Adoption should either have been arranged by that time or the child's parent(s) should be aided financially and with housing and day care sufficiently to enable them to care for their own child. Secondly, nurses and trainees should be assigned babies in such a way as to assure each infant of a continuous relationship to his caretaker(s) while he is placed in the institution.

SUMMARY

The investigation was done to determine the degree to which infant homes designed to be modern and humanized are successful in rearing infants when compared to private families. Twenty-two nursery children and twenty-two family children were given the Hetzer-Buhler developmental tests at ages 6 and 8 months. The two samples were controlled for health, age, sex and social background.

Characterization of the social environment was made on the basis of participant observation, home visits and informal interviews. A remarkable similarity was found in the living environment for all the babies. There were only two marked differences between the life

situation in the nurseries as compared to the private families. The emotional bonds between infant and parental figure appeared less in tense and there was a lack of continuity of the relationship.

At 6 months the probability was greater than 0.5 that the two groups belonged to the same population. The nursery group showed a considerable relative retardation in the 2 months thereafter. It is suggested that the 6 first months of an infant's stay in a nursery be sufficient for arrangement of adoption or sufficient improvement in parent(s) life situation for them to take care of the child.

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STUDIES ON THYROID PROTEINS IN CHILDHOOD GOITRE

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Congenital goitre may be caused by a block in the synthesis of thyroid hormones, e.g. in the step of iodide concentration by the thyroid gland, the step of oxidation of iodide and subsequent organification of the ion, or at the level of formation of iodothyronines from iodo-tyrosines. These postulated defects in the synthesis of thyroid hormones have been deduced from clinical data and indirect biochemical evidence (18). Direct measurements of the concentrations of the enzymes which are thought to participate in these reactions have very rarely been performed. Alternative explanations for the pathogenesis of the congenital goitres have been sought in the synthesis of the iodinated proteins mainly thyroglobulin in the thyroid. During recent years there have been several reports regarding the presence of abnormal proteins such as thyralbumin and iodinated particle bound proteins in congenital goitres from sheep (4), cattle (16) and from some patients with congenital goitres (2, 6, 8).

Biopsy studies performed at an early age before the appearance of nodules in the gland may give a more reliable picture of the pattern of iodoproteins in the goitre than the analyses on nodular goitres which hitherto have been presented. This preliminary report deals with studies on thyroid proteins in two children with a possible defect in the organification of iodine and a child with goitre associated with iodine deficiency.

CASE REPORTS

Case 1

This patient was an 18 day old boy with a symmetrical diffuse enlargement of the thyroid gland. His mother and maternal grandmother had goitres. Deafness was not present in the family. The parents were unrelated and the boy was the first child of a 36 year-old woman who underwent surgery for myoma of the uterus and appendectomy in the third month of gestation. She was treated with allyl stanol and hydroxyprogesterone for imminent abortion in the fourth month of gestation. The child was born at Molndal Hospital after 38 weeks of gestation. Birth weight was 3700 g, length 52 cm. At birth an enlarged thyroid gland was noted and the child was transferred to the pediatric ward. The boy was jittery during the first days of life. Laboratory data including lumbar puncture, calcium and magnesium in serum and repeated blood glucose measurements were essentially normal. The neurological symptoms subsided in a few days when the child was on iv nutrition and oral calcium substitution. The skeletal maturation was slightly retarded; the caudal ossification centre of the femur measured 2 mm as compared with the normal full term value of 5 mm.

The clinical studies of the thyroid function are presented in Table 1. Biopsy for the biochemical analyses was performed on day 18. Treatment with thyroxine was then started. The gland diminished in size and the child's growth and development have been normal during the one year follow up.

Case 2

A 4 year old girl had a goitre with hypothyroidism which was diagnosed at the age of 16 months. Heredity for thyroid disorders was present. The maternal grandfather's mother had a thyrotoxicosis and the maternal grandmother had a goitre since age 12 years. On the maternal side there were also diabetes mellitus and allergic disorders. The paternal grandmother had a juvenile goitre. The grandmother's brother also had a goitre of unknown etiology. No overt hypothyroidism

Table 1 Clinical studies of thyroid function

	Case 1	Case 2	Case 3	Normal range
Sex	male	female	female	
Age at biopsy	18 days	4 years	9 years	
Height cm		101.6	150.3	
Weight kg		15.1	42.6	
Thyroid function tests				
PBI $\mu\text{g}/100\text{ ml}$	3.7 ^a	3.1 ^b	6.5	4-8
TSH $\mu\text{U ml}$ by radioimmunoassay	98 \rightarrow 3.6 ^c		290	<40
Radioiodine uptake ¹²⁵ I isotope and time also stated	35 ¹²⁵ I 4 min	+ b d	45 ¹²⁵ I 4 min	20-50
Perchlorate discharge % of thyroidal ¹²⁵ I	30	+ b d	0	<20
Urinary iodine $\mu\text{g}/24\text{ h}$			26	100-200
Thyroid antibodies				
Thyroglobulin tanned red cell agglutination		Negative ^b	1/1 600	<1/100 ^f
Thyroid cytoplasm immunofluorescence		Negative ^b	1/25	<1/20
Complement fixation			Negative	Negative
Cytological examination	Signs of TSH stimulation no signs of thyroiditis		No signs of thyroiditis	
Pathological examination		Colloid goitre		
And smetric test	Normal	Normal	Normal	

On day one of life normal range 8-12 $\mu\text{g}/100\text{ ml}$ at 24 h The mother's PBI was 8.3 $\mu\text{g}/100\text{ ml}$

^a At the age of 16 months before thyroxine therapy

^b After 3 weeks on thyroxine 50 $\mu\text{g}/\text{day}$

^c Semiquantitative data utilizing ¹²⁵I

^d At 15 months of age before treatment At the time of biochemical study the biopsy showed histological signs of an inactive gland with flattened epithelial cells and follicles of varying size (Dr B. Robertsson)

^e Normal value for adults

was known in any of the families and there was no deafness nor any consanguinity

The mother was treated with hydrochlorothiazide during the pregnancy because of edema. After 36 weeks twins were born in a normal delivery. The patient's birth weight was 2480 g length was 49.5 cm. The dizygotic twin (as judged by different blood groups) weighed 2340 g length 47 cm. When the patient was 2 months of age a swelling of her neck was observed by the mother. The motor development of the patient was somewhat slower than that of her twin sister. She did not walk at the age of 16 months.

At the age of 15 months the child was admitted to the Department of Pediatric Surgery, Kronprinsessan Lovisas Barnsjukhus, Stockholm because of a large left thyroid tumour. Surgical biopsy showed colloid rich thyroid tissue. The child was transferred to the pediatric ward for further investigations. The physical examination showed a girl of normal stature at the 50th percentile. The skin was pale but the child was not distinctly hypothyroid. The thyroid gland was prominent mainly the right lobe where the surgical biopsy had been performed. The surface was smooth and the gland was considered to be diffusely enlarged. She had 7 deciduous teeth (normal value at 16 months $10 \pm 6 \pm 2$ SD). The thyroid function tests are given in Table 1. The biochemical techniques used in this paper were not available and

the child was started on thyroid hormone. During the first 6 months she received only 20 to 40 mg daily of desiccated thyroid. In this period she did not increase in length. She was then put on thyroxine 0.1 mg daily with rapid improvement. The size of her thyroid gland decreased and she started to grow. She started to walk at the age of 23 months. Her psychomotor development was in the low normal region at age 3.5 years. Her audiogram was normal. The thyroid gland was still palpable at 4 years in spite of an adequate substitution therapy as judged by normal growth rate, normal levels of protein bound iodine (PBI) and thyroid stimulating hormone (TSH). Percutaneous thyroid biopsy under general anaesthesia was performed for biochemical and histological analyses during thyroxine treatment before and after 6 days of treatment with TSH.

Case 3

A 9 year old girl was admitted after having had a goitre for 1 month. Her maternal grandmother had an adenomatous goitre. A paternal female relative had an enlarged thyroid gland. There was no known deafness nor any consanguinity in the family.

The patient was the first of two children. Her birth weight was 5.0 kg. Pregnancy and the neonatal period were normal. The past history was uneventful. The psychomotor development was normal. She was

Table 2 Distribution of ^{125}I after *in vitro* incubation for 24 h of the thyroid biopsy specimens in the 3 patients and in human fetal thyroid glands incubated in the same conditions

	Thyroid tissue weight mg	Total cpm ^{125}I in the tissue	^{125}I tissue/medium ratio	of ^{125}I in phosphate ^a extract	of ^{125}I in extract that was not dialysable ^b
Case 1	4.3	7.2×10^3	2.3	96	0
Case 2 while on thyroxine 0.15 mg/d	3.1	2.1×10^4	0.8	90	0
After 5 days of TSH stimulation ^c	$\begin{Bmatrix} 1.0 \\ 3.6 \end{Bmatrix}$	$\begin{Bmatrix} 2.6 \times 10^4 \\ 1.7 \times 10^4 \end{Bmatrix}$	$\begin{Bmatrix} 1.6 \\ 0.6 \end{Bmatrix}$	$\begin{Bmatrix} 83 \\ 92 \end{Bmatrix}$	$\begin{Bmatrix} 0 \\ 2 \\ 2 \end{Bmatrix}$
Case 3	2.6	3.6×10^3	113	91	40
Control maternal fetal thyroid glands			2-28	80-95	5-10
Crown rump length 6-12 cm					
n = 11					
The range is given					

^a 0.02 M sodium phosphate 0.1 M KCl pH 7.4

^b Dialysis against the phosphate buffer for 16+4 h at +4 °C

^c Two biopsies examined

A tall girl growing along the 99th percentile. She had not noticed any tenderness in the neck nor any fever or fatigue. At the physical examination there were no signs of puberty and she was euthyroid. The skin had normal texture and temperature. Finger tremor was lacking. The Achilles tendon reflexes were normal. Her thyroid gland was conspicuously and symmetrically enlarged. The surface was smooth but the gland was distinctly firmer than normal. The results of the laboratory tests of her thyroid function are given in Table 1.

METHODS

Biopsies from the thyroid glands were obtained percutaneously by a rotating needle developed and applied to thyroid biopsies by Ljunggren & Radner (7). The biopsy was performed either in local (prilocain) or general anaesthesia depending on the age of the patient. Two to 5 mg specimens of thyroid tissue were recovered. Where indicated a fragment was immediately immersed in 10% formaldehyde for pathological examination. The remaining tissue was used for biochemical studies as described in a previous paper on fetal thyroid glands (15). In brief, the tissue was incubated for 24 hours at 37 °C in 2 ml of Eagle's medium without leucine containing 200 μCi of ^3H leucine specific activity 15-20 Ci/mmol, 1-10 μCi of carrier free Na^{125}I and 6 mg of benzylpenicillin. The tissues were weighed and the ^{125}I radioactivity was measured in the tissue and the medium. The ratio of the ^{125}I radioactivity per mg tissue/ ^{125}I radioactivity per μl medium was calculated. The soluble proteins were extracted with 1 M KCl phosphate buffer, dialysed and separated by sucrose density gradient centrifugation. Each fraction of the gradient was analysed for ^3H and ^{125}I radioactivities and absorbance at 210 nm

after dilution 1 to 3. Relevant peak fractions were analysed by immunochemical precipitation with rabbit antiserum against human 19 S thyroglobulin as described earlier (14).

Analytical methods

The methods used were serum PBI (1), serum TSH by radioimmunoassay (13), radioiodine uptake employing oral ^{125}I (3). Perchlorate discharge test was performed 70-75 min after *iv* administration of ^{125}I (1 μCi in Case 1, 100 μCi in Case 3) according to Nilsson (11). In Case 1 100 mg of NaHClO_4 and in Case 3 500 mg of NaHClO_4 were administered orally.

Passive haemagglutination tests were performed to titrate antibodies to thyroglobulin. Indirect immunofluorescence and complement fixation techniques were used for detection of antibodies to thyroid cytoplasmic antigen as described (5).

RESULTS

The results of the thyroid function tests are summarized in Table 1. Case 1 was hypothyroid as judged by a low PBI in the neonatal period and an elevated serum TSH which decreased during therapy with thyroxine. ^{131}I uptake *in vivo* was rapid in the thyroid region. Perchlorate rapidly discharged 30% of the accumulated radioiodine indicating a defect in the organification of iodine.

In Case 2 the PBI was low at age 16 months. ^{131}I was accumulated in the thyroid region and

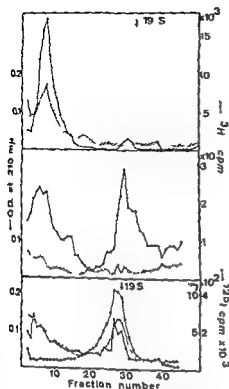


Fig 1 The sucrose density gradient centrifugation profiles of the crude soluble extracts from the thyroid biopsies of Case 1 (top) Case 2 first biopsy before TSH therapy (middle) and Case 3 (bottom). The glands were incubated in the presence of 200 μ Ci of 3 H leucine and 1–10 μ Ci of 125 I. Sucrose gradient 5–30%. Centrifugation for 225 min at 700 000 g. No 125 I radioactivity was detected in Cases 1 and 2. 17–19 S denotes the location of the peak of absorbance at 210 nm of purified adult human thyroglobulin run in the same rotor. Sedimentation is from left to right.

could partially be discharged by perchlorate. This child was clinically possibly hypothyroid. The perchlorate test suggested a defect in the organification of iodine although the technique used only allowed semiquantitative evaluation.

Case 3 was not clinically hypothyroid. The laboratory data showed a normal PBI but an elevated TSH level in the serum. The radioiodine uptake was in the upper normal region. The iodine excretion in the urine was definitely below the normal limit. Antibodies against thyroglobulin and thyroid cytoplasm antigens were present in the serum although cytological aspiration biopsy did not show signs of thyroid

itis. This child could still compensate her failing thyroid gland by an increased TSH stimulation.

Biochemical *in vitro* studies

In Table 2 is shown the 1 I tissue/medium ratio in the biopsies from the 3 patients and from human fetal thyroid glands incubated under the same conditions. Case 1 accumulates radioiodine most of which was found in the soluble extract although as shown in the table no 125 I radioactivity is left in the non-dialysable fraction. This indicated that the gland concentrated radioiodide but did not organify radioiodine *in vitro*. In Case 2 the first biopsy performed while she was treated with thyroxine did not accumulate iodine. After treatment with 1 U of thyrotrophin (Actyron[®], Ferring, Malmö, Sweden) per day for 6 days two biopsies were obtained. At this time there was a slight accumulation of 1 I in one of the specimens. Some 1 I radioactivity remained in the non-dialysable fraction although it was too low to be traced in the subsequent analysis of the soluble proteins by ultracentrifugation on a sucrose density gradient.

In contrast to the first two goitres the thyroid specimen from Case 3 showed a higher 1 I tissue/medium ratio than the fetal thyroid glands. The non-dialysable portion of the radioactivity was also augmented. The data do not permit conclusions on the relative proportions between an increase in the concentration and organification of radioiodine as the 1 I tissue/medium ratio in the experiment includes both processes.

In Fig 1 is shown the result of the sucrose density gradient centrifugation of the soluble proteins from the incubated thyroid biopsies.

In Cases 1 and 2 there was no radioiodine detectable in any fraction. The protein pattern as judged by absorbance at 210 nm showed a low molecular peak but no 17–19 S proteins. 3 H label was present in the slow sedimenting proteins. In both patients there was also a 3 H labelled peak with a sedimentation of 17–19 S as compared with human adult 19 S thyroglobulin run in the same rotor. In the

Table 2 Distribution of ^{131}I after in vitro incubation for 24 h of the thyroid biopsy specimens in the 3 patients and in human fetal thyroid glands incubated in the same conditions

	Thyroid tissue weight mg	Total cpm ^{131}I in the tissue	^{131}I tissue/medium ratio	of ^{131}I in phosphate ^a extract	of ^{131}I in extract that was not dialysable ^b
Case 1	4.3	7.2×10^5	2.3	96	0
Case 2 while on thyroxine 0.15 mg/d	3.1	2.1×10^4	0.8	90	0
After 5 days of TSH stimulation ^c	$\begin{Bmatrix} 1.0 \\ 3.6 \end{Bmatrix}$	$\begin{Bmatrix} 2.6 \times 10^4 \\ 1.7 \times 10^4 \end{Bmatrix}$	$\begin{Bmatrix} 1.6 \\ 0.6 \end{Bmatrix}$	$\begin{Bmatrix} 83 \\ 92 \end{Bmatrix}$	$\begin{Bmatrix} 2 \\ 2 \end{Bmatrix}$
Case 3	2.6	3.6×10^5	113	91	40
Control material fetal thyroid glands Crown rump length 6-12 cm $n=11$ The range is given			2-28	80-95	5-10

^a 0.02 M sodium phosphate 0.1 M KCl pH 7.4

^b Dialysis against the phosphate buffer for 16+4 h at +4 °C

^c Two biopsies examined

■ tall girl growing along the 99th percentile. She had not noticed any tenderness in the neck nor any fever or fatigue. At the physical examination there were no signs of puberty and she was euthyroid. The skin had normal texture and temperature. Finger tremor was lacking. The Achilles tendon reflexes were normal. Her thyroid gland was conspicuously and symmetrically enlarged. The surface was smooth but the gland was distinctly firmer than normal. The results of the laboratory tests of her thyroid function are given in Table 1.

METHODS

Biopsies from the thyroid glands were obtained percutaneously by a rotating needle developed and applied to thyroid biopsies by Ljunggren & Radner (7). The biopsy was performed either in local (prilocain) or general anaesthesia depending on the age of the patient. Two to 5 mg specimens of thyroid tissue were recovered. Where indicated a fragment was immediately immersed in 10% formaldehyde for pathological examination. The remaining tissue was used for biochemical studies as described in a previous paper on fetal thyroid glands (15). In brief the tissue was incubated for 24 hours at 37 °C in 2 ml of Eagle's medium without leucine containing 200 μCi of ^3H leucine specific activity 15-20 Ci/mmol, 1-10 μCi of carrier free Na^{131}I and 6 mg of benzylpenicillin. The tissues were weighed and the ^{131}I radioactivity was measured in the tissue and the medium. The ratio of the ^{131}I radioactivity per mg tissue/ ^{131}I radioactivity per μl medium was calculated. The soluble proteins were extracted with a KCl phosphate buffer dialysed and separated by sucrose density gradient centrifugation. Each fraction of the gradient was analysed for ^3H and ^{131}I radioactivities and absorbance at 210 nm

after dilution 1 to 3. Relevant peak fractions were analysed by immunochemical precipitation with rabbit antiserum against human 19 S thyroglobulin as described earlier (14).

Analytical methods

The methods used were serum PBI (1), serum TSH by radioimmunoassay (13), radioiodine uptake employing oral ^{131}I (3). Perchlorate discharge test was performed 70-75 min after i.v. administration of ^{131}I (1 μCi in Case 1, 100 μCi in Case 3) according to Nilsson (11). In Case 1 100 mg of NaHClO_4 and in Case 3 500 mg of NaHClO_4 were administered orally.

Passive haemagglutination tests were performed to titrate antibodies to thyroglobulin. Indirect immunofluorescence and complement fixation techniques were used for detection of antibodies to thyroid cytoplasmic antigen as described (5).

RESULTS

The results of the thyroid function tests are summarized in Table 1. Case 1 was hypothyroid as judged by a low PBI in the neonatal period and an elevated serum TSH which decreased during therapy with thyroxine. ^{131}I uptake in vivo was rapid in the thyroid region. Perchlorate radioiodine indicating a defect in the organification of iodine.

In Case 2 the PBI was low at age 16 months. ^{131}I was accumulated in the thyroid region and

No radioiodine was detectable in the thyroglobulin present as identified by sucrose density gradient centrifugation. ^3H leucine incorporation and immunochemical precipitation of the ^3H activity with antithyroglobulin serum. The estimated sedimentation coefficient of 17–19 S confirms the data on the sedimentation rate of uniodinated thyroglobulin (12). Quantification of the amount of this protein was not possible with the method used.

No radioiodine was present in any soluble or particle bound proteins. Nor was there any detectable incorporation of tritiated leucine into abnormal proteins as compared to the pattern observed in the fetal thyroid gland (15). The data agree with those of Milunovic et al (9) and Mouriz et al (10) who did not find any abnormal iodoproteins in one respective in 2 patients with nodular goitre, organification defect and deafness (the so called Pendred's Syndrome). Medeiros Neto et al (8) found a particulate thyroid iodoprotein after *in vitro* labelling in one patient with nodular goitre or organification defect and deafness. The varying results may be explained by different pathogenesis in each case. The possible biochemical heterogeneity of the individual nodular goitres that were examined by these authors makes interpretations very hazardous.

The results obtained in Case 2 were disappointing. The biopsies were performed while substitution therapy was given. The thyroid gland was inactive as judged by the histological picture and the poor *in vitro* incorporation of ^1I and ^3H leucine into the thyroid proteins. A 6 day course of TSH therapy only slightly augmented the *in vitro* uptake of radioiodide but failed to increase the ^3H leucine incorporation. We did not feel justified to stop the substitution therapy for a short period of time in this patient at age 4 years because of the slight possibility of causing brain damage. In a 7 year-old patient with congenital goitre and hypothyroidism successfully treated with thyroxine since age 3 months therapy was withheld for 5 weeks until overt hypothyroidism increased TSH levels and the reappearance of

a minute goitre was noted. Biopsies on this patient gave such a low yield that the biochemical analysis was unrewarding (unpublished data). The experience obtained from these 2 patients makes me reluctant to continue refined biochemical thyroid studies on goitres in patients who have been subjected to treatment with thyroxine.

In Case 3 the *in vitro* biochemical findings indicated an augmented uptake and utilization of radioiodine in the thyroid tissue as judged by the increased tissue/medium ^1I ratio and the high percentage of the isotope found in the non-dialysable protein bound fraction of the soluble thyroid extract. ^1I and ^3H were incorporated into thyroglobulin as identified by sucrose density gradient centrifugation. No abnormal iodinated or uniodinated protein was detected. The pathogenesis of the rapidly increasing goitre in this 9 year old girl seemed to be complex. She had an iodine deficiency as judged by her low urinary excretion of iodine. Genetically there was a family history of goitre. Furthermore the tests of autoimmune reactions were inconsistent and did not exclude a thyroiditis. The biochemical findings *in vitro* did not contradict the diagnosis of iodine deficiency.

SUMMARY

Three patients aged 18 days, 4 and 9 years respectively with childhood goitre were studied by labelling *in vitro* with ^1I and ^3H leucine of microbiopsies from the thyroid gland. The soluble thyroid proteins were isolated by means of sucrose density gradient centrifugation.

The first patient was clinically hypothyroid and showed a positive perchlorate discharge test. His thyroid tissue did accumulate iodide but incorporated only ^3H into uniodinated thyroglobulin identified by a sedimentation coefficient of 17–19 S and reactivity to antiserum against human adult thyroglobulin. The clinical and biochemical findings suggest that there was a defect in the organification of iodine with a qualitatively unaltered synthesis of the protein part of thyroglobulin.

Table 3 *Immunochemical precipitation of ^3H and/or ^{125}I labelled human thyroglobulin from Case 1 and Case 3 with rabbit antiserum against human adult thyroglobulin*

Sucrose gradient fractions from the 17-19 S peak were dialysed against the standard buffer. To one aliquot of each sample no antiserum was added (Assay No. 1). To another aliquot was added 20 μl of anti human adult thyroglobulin serum (Assay No. 2). Inhibition of ^{125}I precipitation was measured by using another aliquot containing in addition 2 mg of unlabelled purified adult thyroglobulin (Assay No. 3). After incubation at 37 C for 30 min and at 4 C for 24 h the mixture was centrifuged at 10 000 g for 30 min. The supernatants were measured for either ^3H radioactivity in a liquid scintillation counter or ^{125}I radioactivity in a well scintillation counter (14). As control was used human fetal thyroglobulin obtained from *in vitro* incubation of ^3H leucine with the thyroid gland from a fetus of crown rump length 9.2 cm.

Subject	Total radioactivity per assay		Assay No. 1 Non specific precipitation of radioactivity	Assay No. 2 Radioactivity precipitated by antithyroglobulin serum	Assay No. 3 Radioactivity precipitated in the presence of unlabelled thyroglobulin
	cpm ^{125}I	dpm ^3H			
Case 1		2.5×10^3	10	73	11
Case 3	1.8×10^{10}		1-6	97-100	0
Control		2.1×10^4	13	70	26

* Two samples were tested

biopsy obtained after 5 days of therapy with TSH to Case 2 the ^3H radioactivity did not increase in the 17-19 S region.

In Case 3 a protein peak was present in the 17-19 S region labelled with ^{125}I and ^3H . The slow sedimenting protein peak was labelled with ^3H . There were no ^{125}I and ^3H in any unusual or unexpected area of the gradients as compared with normal thyroid proteins in the human fetus. The peak fraction of the 17-19 S ^3H activity in Case 1 and the 17-19 S ^{125}I peak fraction in Case 3 were used for immunoprecipitation with antithyroglobulin serum (Table 3). Both peaks contained radioactivity that was mainly precipitable with antithyroglobulin serum. The precipitate was not due to unspecific absorption of the radioactivity to the precipitate complex since it could be inhibited by the addition of an excess of unlabelled human 19 S thyroglobulin.

DISCUSSION

This preliminary report presents data on the soluble thyroid proteins in three patients with diffuse goitres during childhood. The biochemical assays were made on microspecimens obtained by rotating biopsies according to the technique developed by Ljunggren & Radner (7). This technique offers distinct advantages

as compared with biopsies obtained at open surgery or biopsies taken by the Silverman technique. It is less traumatic and may be performed on an outpatient basis. The simplicity of the procedure facilitates biochemical studies on infants with a goitre before the thyroid glands has become nodular due to prolonged TSH stimulation or substitution therapy has been instituted.

The *in vitro* technique circumvents the ethical problems involved in the use of radioiodine in childhood (19). The limitations of this biopsy technique depend on the small size of the samples obtained—2 to 10 mg. Analysis of enzymes involved in the synthesis of the thyroid hormones may not be performed. Likewise determination of the amount of different cellular constituents such as microsomes or chemical determination of iodine is not practicable at present. The rapid development of different micromethods may very well extend the applicability of the method. It should be pointed out that studies on biopsy specimens may not be representative for other portions of the thyroid gland (17).

Case 1 had a goitre, hypothyroidism and a positive perchlorate discharge test. Clinically the disease could be classified as an organification defect. The *in vitro* results suggested that the thyroid gland did concentrate iodide

THE INCIDENCE AND GENETICS OF METACHROMATIC LEUCODYSTROPHY IN NORTHERN SWEDEN

A. H. GUSTAVSON and BENGT HAGBERG

From the Department of Paediatrics University Hospital Uppsala Sweden

It has been possible for about 10 years to diagnose metachromatic leucodystrophy (MLD) or sulphatide lipidosis with certainty during life by means of urinary tests (1, 2, 6, 7) or biopsy examination of peripheral nerve specimens (8, 18). With the introduction of tests on the activity of the component aryl sulfatase A of the enzyme cerebroside sulfatase in the urine (3) it has even become possible to reveal the disease in the neonatal period in a pre-clinical stage (5). Very exact enzymatic tests on leucocyte concentrates (14) and cultured fibroblasts (12) have also become available during recent years. Thus the necessary diagnostic tools now exist for mapping down the frequency and the mode of inheritance of this disorder.

The aim of this investigation was 1) to find out the incidence of MLD in the two most northern regions for health and sick care of Sweden (Umeå and Uppsala regions consisting of the counties of Norrbotten, Västerbotten, Västerorrland, Jamtland, Kopparberg, Gävleborg, Västmanland and Uppsala; see Fig. 1) with a total population of about 2 million people and 2) to perform genetic and genealogic studies on the clinical material collected. The study was facilitated by the fact that one of the authors (B. H.) served as the consultant child neurologist for the area in question during the period of the investigation i.e. 1956-1967 and consequently saw and investigated personally

all progressive neurological cases with any suspicion of this disease.

MATERIAL

All children from the Umeå and Uppsala regions for health and sick care who during the period 1956-1967 presented symptoms characteristic or suggestive of MLD were investigated clinically by one of the authors (B. H.). During this period a definite diagnosis of late infantile MLD was established on clinical, histological and specific laboratory evidence in 11 children and another child, a boy, was found to have the juvenile form of MLD.

Family data were collected from hospital records and from interviews with the parents. Genealogic data on the patients and their relatives were collected from the parish records and county archives. In this way information was obtained on 2 additional children with a definite diagnosis of late infantile MLD born in 1953 and 1954 and each belonging to a sibship in the original group. Investigation also revealed a boy with juvenile MLD born in 1945 who was a brother of the above mentioned child with the juvenile type of MLD. Our total series of MLD thus consisted of 13 cases with the late infantile form from 11 sibships (Table 1) and 2 sibs with the juvenile form of MLD. The 2 brothers with juvenile MLD were the 1st and 5th of 5 siblings. The parents were healthy and no other cases of MLD were found among the relatives. The youngest of the two affected brothers, L. K., born February 2nd 1960 was 11 years of age on manifestation of symptoms. The clinical picture combined with pathognomonic histochemical changes in the sural nerve biopsy, a very low activity of urinary aryl-sulfatase A and the specific dihexose sulfatide spot in the urine proved the diagnosis of MLD. The boy is still alive at 10 years of age but is in a poor general condition. The age at manifestation of symptoms in the elder brother, K. A. K., born July 15 1945 was 5 1/2 years.

The second patient was on substitution therapy because of hypothyroidism at the time of the study. The use of similar methods after 1 week of exogenous TSH stimulation did not give any further information regarding the pathogenesis of the goitre.

The third patient was euthyroid, compensating for iodine deficiency with increased secretion of TSH. The biochemical studies revealed an increased in vitro accumulation of radioiodine and a normal synthesis of iodinated thyroglobulin as compared to the human fetal thyroid gland.

No abnormal iodoprotein was detected in any of the patients.

ACKNOWLEDGEMENTS

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Table 1 Family data on the patients with late infantile metachromatic leucodystrophy

M = male F = female

Family number	Number of children		Order in sibship	Sex of affected children	Age at manifestation of symptoms (months)
	Total	Affected			
1	1	1	1/1	F	15
2	1	1	1/1	F	18
3	2	2	1/1 2/2	F F	24 24
4	2	1	2/2	M	15
5	0	1	4/6	M	18
6	4	2	2/4 4/4	M M	24 15
7	3	1	3/3	M	9
8	2	1	2/2	M	15
9	2	1	2/2	M	9
10	1	1	1/1	M	15
11	3	1	3/3	F	13
Total	27	13			

He died at 11 years of age. The clinical picture and course of his disease will be described in detail in a further publication (11). Both cases fall within the age criteria of Moser & Lees (13) for juvenile MLD.

EPIDEMIOLOGY

Age at manifestation and incidence. For the 11 proband cases with late infantile MLD the mean age at manifestation of the disease was 14 months, with a range of 9 months to 2 years. Thus the cases of late infantile MLD born in the Umeå and Uppsala regions for health and sick care during the period 1955-1965 should be almost fully covered by the present material. During this period 8 cases of late infantile MLD were born. The number of births in the Umeå and Uppsala regions during the period 1955-1965 was 316 786. The birth incidence rate for late infantile MLD in that population can thus be calculated to be about 1 per 40 000. Only one case of juvenile MLD was born in the Umeå and Uppsala regions during the same period.

Geographical distribution. When the 15 patients in our total material were grouped according to the county in which they were born it was noted that all were born in the southern part of the investigated area comprising the counties of Jämtland, Kopparberg and Gävleborg (Fig. 1). These counties all border on each other. Six of the 13 children with late infantile MLD and the two sibs with juvenile MLD were born in a fairly restricted area in the central part of Jämtland county where geographical population isolates are common.

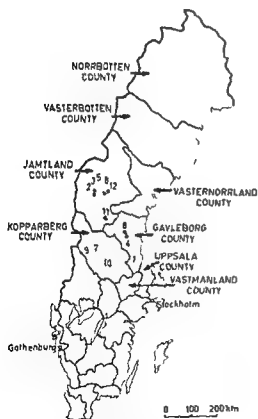


Fig. 1 Map of Sweden. Birth places of children with MLD in the Uppsala and Umeå medical regions are indicated by filled circles. The numbers indicate family numbers referred to in the text.

GENEALOGY

The genealogic data of the patients and their parents were collected from parish register of fices and county archives. Genealogic data were obtained for at least 7 generations back for 6 of the 12 families and for more than 5 generations back for the other 6.

A Late infantile MLD A consanguineous marriage was verified between the parents of each of three probands with late infantile MLD viz. family 5 (Fig. 2) family 10 (Fig. 3) and family 11 (Fig. 4). None of these were first cousin marriages. In the other 8 families with late infantile MLD no indication of consanguinity between the parents was discovered.

The ancestries of families 2, 3, 5 and 6 could be traced back to the years 1690–1730 and to the same central part of Jamtland county as where the probands were born. The ancestry of family 1 was traced back to the year 1730 and to the same village in Gavleborg county as where the proband was born. The ancestors of family 4 were traced back to the year 1700 and to the same village in Gavleborg county where the proband was born.

The ancestors of the father of the proband in family 8—from Gavleborg county—were

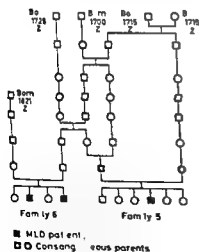


Fig. 2 Pedigrees of families 5 and 6. The birth county is indicated by a letter Z denotes the county of Jamtland.

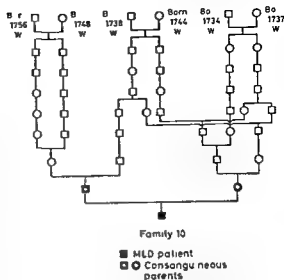


Fig. 3 Pedigree of family 10. The birth county is indicated by a letter W denotes the county of Kopparberg.

traced back to 1710 and came from the county of Jamtland.

The ancestors of family 10 from Kopparberg county were traced back to the beginning of the 18th century and to the same village as where the parents of the proband were born (Fig. 3).

The father of the proband in family 7—Kopparberg county—had common ancestry with the mother of the proband in family 9.

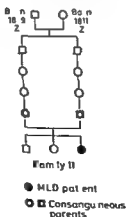


Fig. 4 Pedigree of family 11. The birth county is indicated by a letter Z denotes the county of Jamtland.

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He died at 11 years of age. The clinical picture and course of his disease will be described in detail in a further publication (11). Both cases fall within the age criteria of Moser & Lees (13) for juvenile MLD.

EPIDEMIOLOGY

Age at manifestation and incidence For the 11 proband cases with late infantile MLD the mean age at manifestation of the disease was 14 months with a range of 9 months to 2 years. Thus the cases of late infantile MLD born in the Umeå and Uppsala regions for health and sick care during the period 1955–1965 should be almost fully covered by the present material. During this period 8 cases of late infantile MLD were born. The number of births in the Umeå and Uppsala regions during the period 1955–1965 was 316 786. The birth incidence rate for late infantile MLD in that population can thus be calculated to be about 1 per 40 000. Only one case of juvenile MLD was born in the Umeå and Uppsala regions during the same period.

Geographical distribution When the 15 patients in our total material were grouped according to the county in which they were born it was noted that all were born in the southern part of the investigated area comprising the counties of Jämtland, Kopparberg and Gävleborg (Fig. 1). These counties all border on each other. Six of the 13 children with late infantile MLD and the two sibs with juvenile MLD were born in a fairly restricted area in the central part of Jämtland county where geographical population isolates are common.

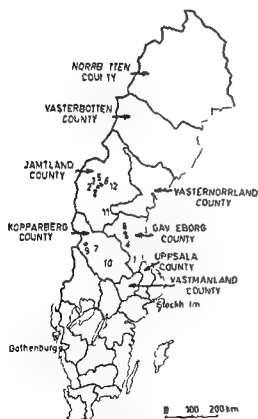


Fig. 1 Map of Sweden. Birth places of children with MLD in the Uppsala and Umeå medical regions are indicated by filled circles. The numbers indicate family numbers referred to in the text.

GENEALOGY

The genealogic data of the patients and their parents were collected from parish register of fices and county archives. Genealogic data were obtained for at least 7 generations back for 6 of the 12 families and for more than 5 generations back for the other 6.

A Late infantile MLD A consanguineous marriage was verified between the parents of each of three probands with late infantile MLD viz. family 5 (Fig 2) family 10 (Fig 3) and family 11 (Fig 4). None of these were first cousin marriages. In the other 8 families with late infantile MLD no indication of consanguinity between the parents was discovered.

The ancestries of families 2, 3, 5 and 6 could be traced back to the years 1690–1730 and to the same central part of Jamtland county as where the probands were born. The ancestry of family 1 was traced back to the year 1730 and to the same village in Gavleborg county as where the proband was born. The ancestors of family 4 were traced back to the year 1700 and to the same village in Gavleborg county as where the proband was born.

The ancestors of the father of the proband in family 8—from Gavleborg county—were

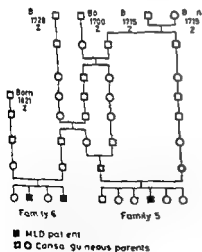


Fig 2 Pedigrees of families 5 and 6. The birth county is indicated by a letter Z, denotes the county of Jamtland.

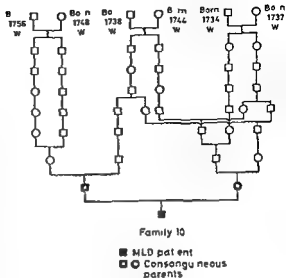


Fig 3 Pedigree of family 10. The birth county is indicated by a letter W, denotes the county of Kopparberg.

traced back to 1710 and came from the county of Jamtland.

The ancestors of family 10 from Kopparberg county were traced back to the beginning of the 18th century and to the same village as where the parents of the proband were born (Fig 3).

The father of the proband in family 7—Kopparberg county—had common ancestry with the mother of the proband in family 9.

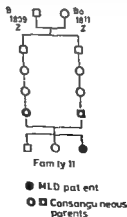


Fig 4 Pedigree of family 11. The birth county is indicated by a letter Z, denotes the county of Jamtland.

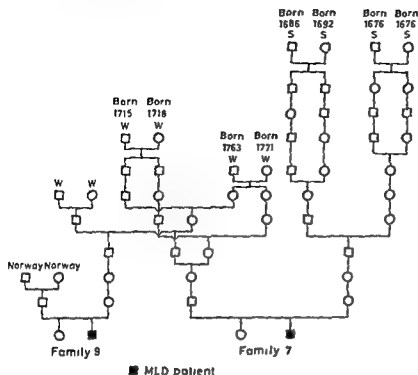


Fig 5 Pedigree of families 7 and 9. Birth counties are indicated by letters W denotes the county of Kopparberg S denotes the county of Varmland

from the same county (Fig 5). Blood relationship was also found between family 5 and 6 from the central part of Jamtland county (Fig 2).

B Juvenile MLD The ancestry of family 12 with juvenile MLD could be traced back 6 generations and to the same village in the central part of Jamtland county as where the proband was born. No indication of consanguinity was discovered between the parents of the affected male siblings and no common ancestor with any of the families with infantile MLD was traced.

GENETIC ANALYSIS OF FAMILY DATA

The present data refer to 22 parents (11 couples) with a total of 27 children (13 of whom (8 boys and 5 girls) were affected with late infantile MLD (see Table 1). The parents were all healthy and no indication of late infantile MLD was found in at least three generations preceding that of the affected children. No association between late infantile MLD and birth rank was found.

As it is most probable that all cases of late infantile MLD born in the Umeå and Uppsala

regions during the period of 1956–1967 were discovered and since the affected siblings were traced independently of the affected proband we applied Weinberg's proband method for the genetic analysis (16). This method gives the result $p=0.20$ which is close to what should be expected from a simple recessive type of inheritance (0.25).

DISCUSSION

No incidence studies on MLD appear to have been reported in literature. The present investigation has shown that the birth incidence rate of late infantile MLD in the northern half of Sweden is about 1 per 40 000. The analysis of the birth places of the patients revealed, however, a geographical cluster in the central part of Jamtland county where population isolates are common. It is of interest to compare these findings with the occurrence of infantile globoid cell leucodystrophy in Sweden. This type of leucodystrophy, which has about the same birth incidence rate as late infantile MLD, is however diffusely spread over the whole country (10).

The present study confirmed that the juvenile

form of MLD is much more rare than the infantile form

Except for a consecutive series of late infantile MLD investigated by Schutta et al (17) only case reports have been published (see Pratt 1967). The series of Schutta et al (17) consisted of twelve sibships and there was a total of eighteen affected and twenty unaffected children. The analysis of this material was suggestive of autosomal recessive inheritance of late infantile MLD though no parental consanguinity was found. The extensive pedigree study with enzyme determinations on heterozygotes by Bass et al (4) of a large family with four patients suffering from late infantile MLD was highly suggestive of autosomal recessive inheritance.

The results of the present investigation also strongly support an autosomal recessive inheritance in late infantile MLD. Affected relatives were confined to sibships; the parents were normal; the sex ratio of affected cases did not differ significantly from unity; parental consanguinity was found in three of the eleven families; the proportion of affected children in the families corrected according to Weinberg's proband method agrees fairly well with what is expected in autosomal recessive transmission.

The concentration of the birth places and ancestry of 6 of the 13 children with late infantile MLD to a certain restricted isolated area of Jamtland county is an indication that the gene could have had its origin in a single gene mutation long time ago. The genealogic analysis has not been able to prove this but one indication is the blood relationship found between two of the families in the isolated district in Jamtland and between two of the families from the county of Kopparberg as well as the ancestor line from the same district in Jamtland in family 8 from Gävleborg county.

The juvenile group of MLD seems to be less homogeneous both clinically, genetically and with regard to age at onset than the late infantile cases. In a few families there appears to be a dominant or X-linked recessive inheritance but in the majority of published cases

autosomal recessive inheritance is suggested (15).

In the family in our series with two brothers affected with juvenile MLD a recessive mode of inheritance, either autosomal or X-linked, appears likely. It has been suggested that the juvenile type of MLD with onset between 4 and 10 years of age represents cases of the late infantile form with a late onset. The parents of the two brothers with juvenile MLD in our series were born in the same isolated part of Jamtland county as 6 of our cases with the late infantile form of the disease. Thus the family lived in the same little Jamtland village with a population of 267 as family 5 with late infantile MLD. However, the genealogic analysis with penetration of 5 generations showed no relationship between the family with juvenile MLD and the families with the infantile form.

SUMMARY

The purpose of this study was to investigate the incidence and genetics of metachromatic leucodystrophy (MLD). A series of 13 cases of late infantile MLD and 2 cases of juvenile MLD was collected from the northern part of Sweden and studied together with their relatives. The series was considered to be adequately representative of the true occurrence of MLD during the period 1955-1965. The incidence rate for late infantile MLD was estimated to be about 1 per 40 000. The MLD patients were highly concentrated to the south-west part of Norrland. From the family analysis it was concluded that late infantile MLD is most probably transmitted by an autosomal recessive gene.

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SHORT COMMUNICATION

TRISOMY 18 IN GREECE

Seven Cases of Pure Trisomy 18 and One with a D/G Translocation

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Trisomy 18 syndrome is now a well defined entity and more than 200 cases have been recorded since the first descriptions by Edwards et al (3) and Patau et al (8) in 1960. The pathology of this multiple anomaly syndrome is attributed to the presence of the extra chromosome 18 but its etiology remains unknown as it is for most other chromosomal disorders.

Warkany et al (10) recently stressed the need for detailed clinical and pathological reporting of cases published because of chromosomal aberrations. The purpose of this article is to present data on 7 newborn Greek infants with this syndrome. These infants were studied at the Cytogenetic Centre of the Institute of Child Health. Three of them were discovered during a screening for genetic disorders of all newborns delivered at the "Alexandra" Maternity Hospital between 1966 and 1968 in a joint study of the Institute of Child Health and the Neonatal Unit of the "Alexandra" Maternity Hospital (7).

These authors reported their data on a study of 10 412 liveborn Greek infants for sex chromatin anomalies. karyotypes performed in all newborns with multiple congenital abnormalities revealed 3 cases of trisomy 18; an incidence of 0.03%. The incidence therefore of the trisomy 18 syndrome in our area does not

differ significantly from the ratio of 1:4500 births observed elsewhere (2, 5).

Clinical and autopsy information on all patients is summarized in Tables 1 and 2. All patients except for case 3 had karyotypes with 47 chromosomes; the extra one being identified as of the E group. The parents' karyotypes were found to be normal. Case 3, however, had 46 chromosomes with an extra 18 but with a balanced D/G translocation. Her mother had normal chromosomes but her father's chromosome study showed a balanced D/G translocation. Blood karyotype data on patients and their parents are summarized in Table 3.

The preponderance of females in our cases (6:1) seems to be in accordance with the increased ratio of affected females to males.

Table 1. Sex, parental age, weeks of gestation and birth weight of patients with trisomy 18

Case no.	Sex	Maternal age at birth	Paternal age at birth	Weeks of gestation	Birth weight (grams)
I	F	43	33	42	1 600
II	M	37	40	43	2 030
III	F	23	30	40	2 400
IV	F	24	42	42	2 020
V	F	33	36	42	3 150
VI	F	25	31	43	1 980
VII	F	41	32	38	1 900

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Table 2 Anomalies observed on 7 cases of trisomy 18

Anomaly	Cases						
	I	II	III	IV	V	VI	VII
Single umbilical artery	-	-	+	-	?	-	?
Apparent mental deficiency	+	+	+	+	+	+	+
Prominent occiput	+	+	+	+	+	-	+
Low set malformed ears	+	+	+	+	+	+	+
Eyes ptosis & inner epic folds	-	-	-	-	-	-	-
Small palpebral fissures	+	+	+	+	+	+	+
Corneal opacities	-	-	-	-	-	-	-
Micrognathia	+	+	+	+	+	+	+
Narrow palatal arch	+	+	+	+	+	+	+
Small oral opening	+	+	+	+	+	+	+
Cleft lip or palate	-	-	-	-	-	-	-
Fingers flexed	+	+	+	+	+	+	+
Index overlaps 3rd/5th over 4th	+	+	+	+	+	-	+
6 or more low arches	?	?	+	+	?	+	+
Simian crease	-	+	-	+	?	+	+
Single crease 3th finger	-	+	-	+	?	-	+
Hypoplastic fingernails	-	-	-	-	-	-	-
Hypoplastic thumb	-	-	+	-	-	-	-
Short hallux	+	+	+	+	+	+	+
Rockerbottom feet	+	+	+	+	+	+	+
Equinovarus	+	+	+	+	+	-	-
Syndactyly 2nd-3rd toe	-	-	+	-	-	-	-
3rd sternum	+	+	+	+	-	-	+
Wide spaced nipples	+	+	+	+	+	+	+
Small pelvis	+	-	+	+	+	+	+
Limited hip abduction	+	-	+	+	+	+	+
Anal anomalies	-	-	-	-	-	-	-
Cryptorchidism	-	+	-	-	-	-	-
Prominent clitoris	+	-	+	-	-	-	-
Gonadal dysgenesis	-	-	-	-	-	+	?
Abnormal uterus	-	-	-	-	-	-	?
VSD	-	+	+	+	-	+	?
ASD	-	-	+	+	-	-	?
PDA	-	-	+	+	+	-	?
Double ureter	-	+	-	-	-	-	?
Meckel's diverticulum	-	-	+	+	-	-	?
Inguinal hernia	-	+	-	-	-	-	-
Abnormal lobation of liver	+	+	+	-	-	+	?
Hypoplastic gallbladder	-	-	-	-	-	+	?
Kernicterus	-	-	-	-	+	-	-

Table 3 Blood cytogenetic data (1) (Method of Moorhead et al (6))

Case no	Patient	Father	Mother
I	47 XY 18+	46 XY	46 XX
II	47 XY 18+	46 XY	46 XX
III	46 XX 18+ D G t (DqGq) + pat	45 XY D G t (DqGq) +	46 XX
IV	47 XX 18+	46 XY	46 XX
V	47 XX 18+	46 XY	46 XX
VI	47 XX 18+	46 XY	46 XX
VII	47 XY 18+	46 XY	46 XY

requires confirmation by collection of large abortion material statistics

The effect of trisomy 18 is almost always lethal in early infancy, although a small number of individuals survived beyond this age. All, but one, of our cases died within the first month of life.

The only factor possibly related to the etiology of this syndrome is a maternal age effect. 4 of the 7 mothers of our patients were 33 years or older. These figures agree with Smith's (9), who estimated that 52.5% of the mothers of trisomy 18 patients were over 35 years old. This suggests as a causative factor of the syndrome, either a defective ovum, because of some pre-conceptive ill effect or just non-disjunction due to an aging ovum. As for case 3, where a balanced translocation existed in the father, it would be interesting to know whether this chromosome anomaly could have caused non-disjunction on another autosome.

Except for the mother of case 6 who since the twentieth week of gestation took Diphenal¹ for control of epileptic seizures, no drugs were taken, no X-rays were performed and no infections during pregnancy were reported. Most of our patients were born after a gestation of more than 40 weeks with a low birth weight (less than 2500 g). The average birth weight of our patients was 2154 g which agrees with the average weight (2183 g) of 90 cases of trisomy 18 analysed by Warkany et al (10).

Combination of Sodium Diphenylhydantoinate and Phenylthylbarbituric acid

found by Ferguson Smith (4) and Weber (11). Although these authors explain this ratio by proposing a greater mortality of males during the period shortly after birth, we believe that this explanation should be valid for the intra-uterine mortality of defective males, rather than in the neonatal period. This of course

The variability of the clinical picture in trisomy 18 can be compared with other syndromes of multiple congenital anomalies manifesting themselves as spectra of congenital malformations. This makes necessary confirmation of the diagnosis on etiological grounds. Nonetheless it is important to collect data from large series as a possible breakthrough to the etiology of the syndrome.

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CASE REPORT

A VARIANT FORM OF BRANCHED CHAIN KETO ACIDURIA

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Branched chain keto aciduria (BCKA) also called maple syrup urine disease is a neuro-metabolic disorder caused by inability to metabolize the branched-chain amino acids leucine, isoleucine and valine, which with their corresponding keto and hydroxy acids, accumulate in all the body fluids. The enzyme defect is localised at the level of the oxidative decarboxylation of these keto acids. The disease may take an acute form leading if not promptly treated, to rapid and irreversible neurologic damage. There is also an intermittent form producing symptoms at a later age and only under special provocative conditions such as fever, infections, vaccinations and excessive protein intake (1, 4, 7, 9, 11). Ketoacidotic attacks appear suddenly and can be rapidly fatal unless diagnosed correctly and given urgent temporary dietary treatment. Patients with this intermittent form of BCKA show a normal mental development and the neurological abnormalities are present only during attacks.

Recently, 2 cases have been described with yet another clinical picture. Schulman et al (13) reported a case of permanent BCKA in a moderately retarded girl of 1 1/2 years showing no neurological abnormalities and no attacks of severe acidosis. Gerritsen et al (6) during routine screening discovered a moderately retarded girl of 18 years with severe

BCKA and slight hyperamino aciduria who also had no neurological symptoms.

In the case reported here a boy was admitted to hospital at the age of 7 months with signs of BCKA but with no history of previous illness. At the time his disease was recognized he showed a severe mental retardation. The institution of dietary treatment resulted in a favorable clinical course so that at the age of 3 years the boy's mental development corresponded with his age.

CASE HISTORY

W. was first admitted to the hospital for vomiting at the age of 7 months, 10 days after a smallpox vaccination. The past history was uneventful.

Examination revealed a pale, semicomatose infant in a dehydrated state. His weight was 6280 g. He had a metabolic acidosis; the pH of the blood was 7.17 and the standard bicarbonate concentration 10.6 mEq/l. The CSF contained 9/3 lymphocytes/mm³ and 31 mg/100 ml of protein. The EEG showed a slight disturbance of the left hemisphere. Intravenous infusions of sodium bicarbonate were started and the metabolic acidosis was corrected in 60 hours. The patient gradually recovered and was discharged well. The BCKA syndrome remained unrecognized.

The boy was readmitted to the hospital at 9 months for a respiratory infection. He was pyrexial and his sedimentation rate was 35 mm. The total plasma protein concentration was normal. The boy's blood glucose was 49 mg/100 ml. The CSF now contained 1/4 lymphocytes/mm³ and 17 mg/100 ml of protein. During his admission at the age of 11 months a transient ataxia was noticed.

Table 1 Urinary amino acids (mg per g creatinine) of patient IV during admission

Age (months)					Treatment started	
	9	10	11	17	II	Normals n=10
Valine	71	67	117	58	56	—
Allo-isoleucine	10	18	11	20	—	—
Isoleucine	31	90	58	33	30	<10
Leucine	31	114	212	62	40	<41

A two-dimensional paper chromatogram showed increased urinary branched chain amino acids. Their corresponding keto acids were also present. These abnormalities highly suspect for BCKA became more pronounced in further examinations. A peculiar odor of the urine was also noticed when the patient had a gastro-er-eritis.

Table 1 shows the urinary branched chain amino acids analysed by quantitative column chromatography according to the Technicon 22 hour method. The excretions were moderately increased or still normal. The increase is less impressive than is usual in patients with the classical form of BCKA. Allo-isoleucine, a characteristic product in this disorder, occurred in all samples until treatment was started. In the plasma only branched chain amino acids were increased (Table 2) suggesting that some form of BCKA might be present.

At the age of 17 months before dietary treatment was started the patient had a severe ataxia. The EEG was abnormal (paroxysmal and bilaterally synchronous delta waves and once a sign of a more local left fronto-temporal disorder). At this time the boy was examined by a psychologist who reported severe developmental retardation (Table 3).

Dietary treatment. We decided to treat this patient with a diet low in branched-chain amino acids. According to the directions of Ireland (8) one of the

nitrogen containing components of this diet consisted of 3 parts of gelatin and 1 part of albumaid (mixture 1). The other nitrogen-containing component (mixture 2) contained amino acids only (Table 4). The complete composition of the diet used when the treatment was started is shown in Table 5.

At first the administration of the diet raised problems because the patient partly refused mixtures 1 and 2. Gradually these difficulties disappeared. Three weeks after starting dietary treatment when the patient was 18 months old analysis of the plasma and urinary amino acids showed a striking improvement (Tables 1 and 2). However the plasma concentrations of methionine, tyrosine and phenylalanine were on the low side. For this reason 400 mg phenylalanine and 800 mg methionine were added to the daily ration. A month later valine was normal but leucine and isoleucine were somewhat low (Table 2). Methionine and tyrosine were too high. Three weeks later the concentrations of methionine and tyrosine became low. A further addition of 100 mg of methionine and also 900 mg of tyrosine was made to the daily diet. The daily intake of mixture 2 was also increased to 265 g. At the age of 21 months increased plasma concentrations of leucine, valine and isoleucine were again found and allo-isoleucine reappeared. This was probably due to excessive protein catabolism since the patient had a respiratory infection and vomited at this time. The plasma methionine level was low but the tyrosine and phenylalanine levels were in the normal range. Once again in

M. C. Janßen of the Department of Psychiatry, Dijkzigt Ziekenhuis Rotterdam.

Table 2 Plasma amino acids (mg per 100 ml) of the patient II

Age	Before treatment 17 (mo)	After start of treatment									Normal (mean + 2 S.D.)
		18 (mo)	19 (mo)	19½ (mo)	21 (mo)	23 (mo)	26 (mo)	31 (mo)	3/18 (yr)	3/12 (yr)	
Valine	14.6	1.75	1.18	2.06	3.64	4.65	2.56	2.40	1.84	1.88	3.63
Allo-isoleucine	1.35	—	—	—	trace	trace	—	—	—	—	—
Isoleucine	6.4	0.59	0.18	0.53	1.66	1.63	0.76	0.95	0.94	0.91	1.16
Leucine	15.1	1.78	1.00	1.90	4.06	4.59	2.30	2.70	2.90	2.8	2.79
Methionine	0.8	0.16	3.25	0.15	0.17	0.48	—	—	—	—	0.49
Tyrosine	0.98	0.38	3.34	0.52	0.6	1.01	—	—	—	—	1.4
Phenylalanine	1.15	0.6	1.46	0.77	0.83	1.44	—	—	—	—	1.28

creased plasma concentrations of branched chain amino acids were observed at the age of 23 months the patient had another 3 days' attack of vomiting and loss of weight caused by an infection. No other symptoms were seen. No further abnormalities have appeared up to the time of writing.

Development after starting dietary therapy

Soon after dietary treatment was instituted the vomiting disappeared. Two months later when the patient was 20 months old (Fig 1) the EEG was normal.

The psychologist reported a favorable progress of motor function and social behavior. At 28 months the psychological evaluation revealed that the retardation of the motor function was disappearing (Table 3). At 3 years the boy's mental development corresponded with his age. His speech however was still retarded but this defect could not easily be evaluated as his brothers also had transient speech difficulties. This defect may possibly have no direct connection with BCKA.

The patient's physical development was satisfactory. After his discharge from the hospital there was a satisfactory gain of height and body weight. At the age of 3 years and 9 months his height was 101 cm (50th percentile) and his weight 16.3 kg (50th percentile). Occasional respiratory infections occurred and once he was readmitted for a gastro-enteritis. However the clinical symptoms of BCKA did not reappear.

Examination of the family

The patient W was the youngest of the 5 children of healthy parents. A sister M (third child) died in

Table 4 *Composition of the protein and amino acid mixtures 1 and 2 used for treatment*

	Mixture 1 Albuminoid Gelatin (mg/g)	Mixture 2 Amino acids (mg/g)
Alanine	65.0	—
Glycine	181.2	353
Proline	120.3	—
Glutamic acid	92.6	353
Aspartic acid	56.0	—
Serine	32.4	—
Threonine	20.4	20.6
Cystine	5.2	17.6
Methionine	8.5 (1)	41.2 (dl)
Tyrosine	8.2	61.8
Phenylalanine	21.7	41.2
Histidine	8.8	32.3
Arginine	66.1	—
Lysine	32.6	59
Tryptophan	17.0	20.6
Leucine	33.5	—
Isoleucine	18.0	—
Valine	27.1	—

coma at the age of 21 months. She had a respiratory infection with vomiting and developed a metabolic acidosis which could not be corrected. A peculiar odor of the urine was noticed. Probably she had the same disease as her brother W. The fourth child suffered from epilepsy but was otherwise healthy and was mentally normal as were the two eldest children.

Heterozygotes can be detected by direct estimation of the decarboxylase activities in peripheral leucocytes using isotope techniques (3, 4, 7). Oral leucine loading tests may also be informative (10, 13, 16). The reliability of both methods has to be further evaluated and it is still uncertain to what extent the 2 methods agree. The latter method may throw more light on the metabolic defect in the whole subject while the former is more closely connected with the typical enzymic system of a special cell.

Leucine tolerance tests were performed on the present patient's family. 150 mg of L-leucine per kg being given by mouth. As can be seen from Table 6 all members of the family tested had an abnormal curve according to the criteria of Woody & Harris (16). Presumably all are heterozygous carriers. Leucine intolerance was more pronounced in the father than in the mother. Similarly the abnormal gene seemed to be more active in the two brothers than in the patient's sister.

The family's pedigree is shown in Fig 2. The female parent in the fourth remove from the father's side was a sister of the male parent in the fourth remove from the mother's side.

It is also noteworthy that a sister of the patient's mother married a brother of the patient's father. Their 7 children remarkably enough are all in good health and mentally normal.

Table 3 *Psychological examination of W*

(1) Tested with the Buhler-Het er infant test 1953^a

- Age 1; 5+17 Developmental quotient 11.80
Motor development low
- Age 1; 9+1 Developmental quotient 0.83
Motor and social improvement
- Age 2; 4+21 Developmental quotient 0.81
Motor and social development
approximately in accordance with age

(2) Tested with the Stanford-Binet test Dutch version described by L. M. Terman & M. A. Merrill Measuring Intelligence 1955 type L

- Age 2; 4 Mental age cannot be determined
Fails on all verbal items of II and II 6
Can build and complete formboard
- Age 3; 10 Mental age 3; 5
IQ 91
Fails on verbal items
Drawing and building in accordance
with age
- Age 4; 10 Mental age 4; 11
IQ 102
Retardation abolished
No typical outlying values in profile

Table 5 Composition of the diet of patient W (11.3 kg) at start of therapy

Leucine 90 mg/kg isoleucine 55 mg/kg valine 71 mg/kg Calories 1321

	Leucine (mg)	Isoleucine (mg)	Valine (mg)	Protein (g)	Fat (g)	Carbohydrate (g)
4 amaret biscuits	23	18	18	0.5	4	44
45 g margarine	—	—	—	—	37	—
40 g jam	—	—	—	—	—	24
2 fruits	—	—	—	—	—	20
50 g potatoes	60	55	50	1.0	—	10
60 g vegetables	56	44	36	1.0	—	2
50 g sugar	—	—	—	—	—	50
80 ml cream 40	70	79	46	1.0	16	—
74 g mixture 1	804	432	650	(22)	—	—
4 g mixture 2	—	—	—	(2)	—	—
515 g mineral supplement (Ireland) ^a	—	—	—	—	—	—
vitamins normal administration	—	—	—	—	—	—

^a 100 g mineral mixture contained 17.6 g Ca 1.3 g K 0.63 g Na 0.31 g Mg 0.14 g Fe 0.019 g Zn 0.015 g Cu 0.013 g Mn 1.2 mg I 0.3 mg Mo 0.2 mg Co 0.04 mg Al 71.2 g lactate 2.8 g Cl 0.97 g P 1.53 g SO₄

DISCUSSION

Our knowledge of the enzymes of the branched chain alpha keto acid dehydrogenase systems is still too incomplete for a quantitative interpretation to be made of the metabolic patterns in BCKA. The accumulation of leucine and its metabolites is greater than that of other branched-chain compounds; this has been interpreted as indicative of separate dehydrogenases. However, other factors must also be

considered such as the inhibition of enzyme activity by related keto acids and the coinduction of dehydrogenases by related amino acids. Both factors have been studied extensively (14). In the rat, two superimposable mechanisms seem to be operative in modulating the dehydrogenase activities: adaptation to the dietary protein intake and a daily activation-deactivation cycle. Bowden & Connelly (2) obtained evidence of the existence of a single enzyme complex for the oxidative decarboxylation of both alpha keto-isocaproic acid (derived from leucine) and alpha keto-beta methyl valeric acid (derived from isoleucine) and a separate enzyme complex for the oxidative decarboxyla-



Fig. 1 The patient at the age of 20 months

Table 6 Leucine tolerance tests on patient's parents, brothers and sister

Plasma leucine in mg/100 ml after an oral load of 150 mg l-leucine per kg bodyweight

	Fasting	1 h	2 h	3 h
Mother	2.7	9.4	12.5	10.9
Father	2.9	12.8	17.0	13.5
Sister aged 9 y	1.7	13.6	9.4	3.9
Brother aged 7 y	2.9	16.6	1.5	7.9
Brother aged 5 y	2.9	13.1	10.4	6.0
Max. values in 5 normals				
Woody & Harris (16)	2.5	10	7.7	6.5
Normal child (analysed in author's laboratory)	1.40	6.83	2.83	2.32

tion of alpha keto isovaleric acid (derived from valine) in bovine liver Goedde et al (7) however, argued for the existence of 3 different decarboxylating enzymes for each of the branched-chain keto acids. The experimental results of Wohlhueter & Harper (14) may be interpreted in 2 ways either a single enzyme complex is responsible for the oxidation of all three branched chain keto acids, or if there are multiple enzymes they are subject to a very exacting co ordination in their regulation.

Whatever the detailed structure of the enzyme systems may be there is strong evidence that in classical BCKA the enzyme defect is complete or nearly complete whereas in the intermittent form and other variants decarboxylase activities are less severely reduced (3, 6, 7, 13).

Classical BCKA is transmitted as an autosomal recessive defect. The decarboxylase activities in the leucocytes of both parents of patients with this classical form are reduced (3). According to Dancis et al (4) the intermittent form is probably also a distinct genetic trait. In the leucocytes they have shown a decrease of decarboxylase activities in the fathers but not in the mothers of the affected children. It is interesting to note that our loading experiment showed that intolerance to leucine was more pronounced in the patient's father than in his mother. Also in both male siblings tolerance seemed to be less than in the patient's sister.

Goedde et al (7) found that in intermittent BCKA either the female or the male parents can have decreased leucocyte decarboxylase activities. They speculated on genetic allele models as a basis for the interpretation of the biochemical patterns in the patients and parents. We must question however whether the leucocyte enzyme activities as they were determined until now, can really reflect genetic structures. The highly variable findings obtained in the same patient by the latter authors suggest the contrary.

The clinical aspects of classical BCKA have recently been discussed by Gauli (5). Attacks

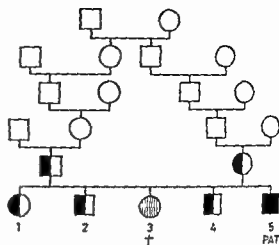


Fig 2 Pedigree of the patient's family ■ presumed homozygous male for variant of BCKA ■ □ presumed heterozygous male and female □ □ not tested

of this disease can be successfully treated with peritoneal dialysis as has been reported by Rey et al (12). Gauli concluded that a diffusible "toxic factor" is responsible for the reversible neurological changes in this disease but this need not necessarily be the branched chain keto or amino acids. The findings of Geritsen & Milton (6) and Schulman et al (13) support this view: their patients who permanently produced branched chain keto-acids did not show any neurological abnormalities. Peritoneal dialysis may also be effective in severe attacks of intermittent BCKA which can be difficult to correct. (In our patient the metabolic acidosis was corrected only after 60 hours.)

The clinical picture of our patient roughly corresponded with that of the intermittent form of BCKA except that his mental development was clearly retarded at the time that diagnosis was made. It was this alarming phenomenon connected rather with structural changes in the brain than with an acute intoxication that prompted us to continue the dietary treatment and not to stop the treatment as soon as the branched chain keto acidotic attack was over, as is usually advised. In this way we hoped to avoid any further episodes of branched chain keto aciduria which might have aggravated the presumed brain-damage. This danger existed for frequent respiratory infections oc-

curred during treatment. Moreover the patient accepted the diet very well and the treatment did not raise any difficulties at home. We realize however that the favorable course of the boy's mental development is not necessarily a result of our prolonged treatment.

SUMMARY

A case of branched-chain keto aciduria is described. The boy's clinical picture was similar to that of patients with the intermittent form of this disorder but severe mental retardation was present.

It proved possible to treat him successfully with a diet low in branched-chain amino acids and at the age of 3 years after 18 months treatment his mental development corresponded with his age.

Details are given of the clinical course and the dietary treatment. The findings in the patient's family are also described with the results of oral loading tests with L-leucine performed on all members of the family.

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Key words Variant forms of branched-chain keto-aciduria, maple syrup urine disease, mental development, dietary treatment, leucine intolerance, family screening.

LETTER TO THE EDITOR

To the editor

I am writing in response to the report by O Schweisguth et al, 'Bilateral Non Functioning Thecoma of the Ovary in Epileptic Children Under Anticonvulsant Therapy in *Acta Paediat Scand* 60 6-10, 1971

The authors have not demonstrated the ovarian lesions to be thecomas. It is likely that the changes in the ovary are a result of partial or intermittent venous or lymphatic obstruction. There are two reasons why the lesions of the ovaries are not thecomas. First, bilateral thecomas as we found in a large study (*Cancer* 21 255 1968) are medical curiosities and reports of bilaterality are misleading and overstate the frequency of it. Second, I reviewed the ovary slides in the second case, and found no evidence of thecoma. There was swelling marked edema with focal hemorrhage but most important, retention of the outline of the ovary and preservation of follicular structures. These findings indicate the lesions are ovarian enlargements not thecomas and are probably secondary to venous or lymphatic obstruction. Such changes usually occur in children secondary to torsion. The literature is beginning to reflect these observations (*Obstet Gynec*, 34 565 1969 and *Amer J Surg*, 117 726, 1969).

I am incorrectly quoted on page 9 of the article as rendering a diagnosis of 'thecal hyperplasia'. In my correspondence with the authors on 3 April 1970 I stated:

"The ovarian changes represent a rare alteration of the ovaries that occurs in association with torsion of the ovarian adnexal structures. The ovaries become tremendously enlarged secondary to venous obstruction, there

is marked stromal edema, stromal hemorrhage and thrombosis of capillaries and veins. The lesion is nearly always found in a child, as is expected from the higher position of the ovaries in the pelvis with greater freedom of motion. These ovarian changes have been regarded by some as torsion of a thecoma but the absence of any well defined outline of a neoplasm and retention of the ovarian outline (as seen in the smaller of the submitted sections) indicates that most are simply ovarian enlargements secondary to venous obstruction. A copy of your gross description would be helpful in completing the pathological details of this interesting case."

Regardless of the exact nature of the ovarian changes in epileptic children taking anticonvulsant medication, the association is most interesting and the authors have performed a service by reporting it.

Sincerely,

Henry J Norris, MD
Chief Gynecologic, Obstetric
and Breast Pathology
Washington DC
USA

The Editor has asked Dr Schweisguth to comment on Dr Norris' letter to the editor.

As long as the possible relationship between anticonvulsant therapy and the ovarian lesions is not determined, the true nature of these thecomas is debatable.

But 1) The diagnosis of thecoma was confirmed in our first case by several trained pathologists as well as by Faber's pathologist

in the case reported by him. The finding of fat in the cells was an argument favoring this diagnosis.

2) To say that the ovarian lesions are associated with torsion and are due to venous obstruction is—at least in our cases—a pure hypothesis which is not based on any surgical or gross pathological examination.

To conclude we have added “non functioning” to the term of “thecoma” in order to emphasize the unusual presentation of these ovarian lesions. We agree that further knowledge in the physiopathology is needed before a true nomenclature can be ascertained.

Odile Schweisguth

PROCEEDINGS OF PAEDIATRIC SOCIETIES

FINNISH PAEDIATRIC SOCIETY

Meeting Febr 13, 1971

Martin Seip (Oslo, Norway) *Fat and carbohydrate metabolism in generalized lipodystrophy*

The most striking feature of generalized lipodystrophy is the extreme paucity of fat in adipose tissue, resulting in a great reduction in the size of the functioning adipose organ. The lack of an adequate adipose organ leads to serious disturbances in fat and carbohydrate metabolism and, in most patients, to a markedly increased metabolic rate in the absence of hyperthyroidism. These disturbances have been studied in 6 patients with the congenital form of generalized lipodystrophy.

The fundamental defect in fat metabolism is the inability to store triglycerides in the adipocytes. As a result a marked tendency to hypertriglyceridemia with increases of the pre- β and chylomicron fractions, fatty infiltration of the liver and some deposition of fat in the reticuloendothelial system can be demonstrated. Great fluctuations in plasma triglycerides are found depending on the dietary supply of fat and carbohydrate. The peripheral utilization of fat is not impaired, plasma FFA low, normal or decreased, plasma glycerol 3-6 fold increased.

The alterations in carbohydrate metabolism seem to be secondary to the greatly impaired ability to store glucose as fat in the adipocytes. During the first years of life glucose tolerance is normal, but from 8-10 years of age

glucose tolerance rapidly decreases, and with cessation of growth at approximately 12 years of age frank diabetes without ketosis develops. Some degree of insulin resistance is demonstrable already from infancy, and the insulin resistance increases with age. The fasting plasma insulin levels are high especially in later childhood and the insulin responses to glucose and tolbutamide stimulation are exaggerated and prolonged. After cessation of growth a partial exhaustion of the β cells seems to occur with somewhat lower fasting insulin levels and a relatively poor response to glucose stimulation. Other aspects of carbohydrate metabolism (peripheral utilization of glucose, hepatic lipogenesis from glucose etc.) are not impaired.

The hyperinsulinism combined with the availability of an excess of calories promotes anabolic processes resulting in increased rate of growth and skeletal maturation, muscular hypertrophy and splenomegaly.

The hypermetabolism may perhaps be due to the ability of fatty acids to uncouple oxidative phosphorylation.

These findings demonstrate most dramatically how important the normal adipose tissue is and the serious consequences of an inadequate adipose organ for a number of metabolic processes.

O Koskimies

PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

Ninth Annual Meeting Lyon July 16-21 1970

H Greenberg P Czernichow R C Reba J Tyson & R M Blizzard (Baltimore Maryland)
Observations on the maturation of thyroid function in early fetal life

Serum from twenty one normal human fetuses was obtained after therapeutic abortion for psychiatric indications. Fetal crown-rump length ranged from 5.2 cm to 22.5 cm corresponding to the gestational age of 65 to 168 days.

Serum thyroxine assayed by the Murphy Pace method was identified in the second smallest fetus examined at 78 days gestation. Thereafter it increased rapidly, maintaining a linear relationship with crown-rump length until term ($r=0.80$ $p<0.001$). A similar rise was seen in TBG capacity ($r=0.841$ $p<0.001$) and in the affinity of TBG for tracer amounts of ^3H -T₄ ($r=0.841$ $p<0.001$). Free thyroxine also increased in a linear relation to gestational age until reaching term levels at 18 to 20 weeks ($r=0.903$ $p<0.001$). This increase occurred despite a rapid decrease in the dialysable fraction of thyroxine during this period.

Radioimmunoassayable TSH was detected at 78 days gestation thereafter increasing rapidly to reach term levels by 16 weeks gestation. After 100 days gestation levels were usually

40 $\mu\text{U/l}$ higher than that seen in normal infants and children. TSH did not correlate with total serum thyroxine but did correlate with free thyroxine in fetal sera.

A significant fetal to maternal gradient of free thyroxine was demonstrated in fetuses less

than 20 weeks gestation. Fetal TSH levels were found to exceed that of the corresponding maternal TSH throughout most of the gestational period examined.

P Olin S Almquist & R Ekholm (Stockholm)
Human fetal thyroid gland. Influence of propylthiouracil and of pituitary thyrotrophin

Previously we have shown *in vitro* synthesis of thyroxine and thyroglobulin in the thyroid glands from 10-11 week old human fetuses. Prior to that time no iodide was incorporated into the thyroid although a thyroglobulin like uniodinated protein appeared at a somewhat earlier stage.

The present studies showed that in the presence of a reducing substance as propylthiouracil 0.01 M radioiodide was concentrated 5-20 times into the thyroid tissue from fetuses above age 11 weeks without any iodination of thyroglobulin. Protein synthesis as judged by ^3H leucine incorporation remained unaltered. Thus the concentrating mechanism for iodide had developed at the time when the formation of thyroglobulin occurred.

The *in vivo* effects of carbimazole on the human fetal thyroid gland was studied when a thyrotoxic woman on carbimazole treatment was subjected to a therapeutic abortion. The thyroid glands from the twin fetuses, crown-rump length 105 and 120 mm, were normal judged by their light and electron microscopic morphology as compared with our observations on normal fetuses of corresponding age. The

Table 1 *The conversion of androstenedione and dehydroepiandrosterone to testosterone by foetal testes in the presence of oxymetholone*

Foetal age (weeks)	Conversion to testosterone (dpm)			
	From AD	From AD + Oxy metholone	From DHA	From DHA + Oxy metholone
6	6 828	6 253	10 618	7 653
8	8 586	1 135	20 811	3 390
8	14 552	6 261	50 170	7 432
9	5 285	2 384	11 096	6 826
12	6 568	3 133	18 115	3 680
22	50 143	3 770	42 358	16 389

in vitro incubation showed a normal synthesis of iodinated thyroglobulin. Thus at fetal age 14–15 weeks the drug did not influence the structure of the thyroid nor inhibited the synthesis of thyroglobulin *in vitro*.

In order to clarify the relationship between the pituitary gland and the synthesis of thyroglobulin the pituitary content of thyrotrophin was determined in a double antibody radioimmunoassay. Thyrotrophin was demonstrated in four fetuses 8–9 weeks old and in increasing amounts in fetuses more than 11 weeks old. Thus pituitary thyrotrophin appears at the same time or earlier than the biosynthesis of thyroglobulin.

W Hamilton (Glasgow) *The action of oxymetholone on human foetal tests*

Foetal testes were incubated with androstenedione (AD) and dehydroepiandrosterone (DHA) according to Hamilton et al (1). To the flask containing the right testis of each pair oxymetholone was added in the same concentration as the substrates, while the left testis of each pair served as a control. In Table 1 the inhibitory effect of oxymetholone on the synthesis of testosterone from both AD and DHA is shown. Thus, in keeping with other C2 substituted C19 compounds (2), oxymetholone blocks 3β hydroxysteroid dehydrogenase and 17β reductase. The favourable anabolic/androgen ratio of oxymetholone as an anabolic hormone might be due to a similar effect *in vivo*.

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N Jossio (Paris) *Histo enzymatic study of the human fetal testis*

A histo enzymatic study of testicular tissue was made in fifteen fetuses whose gestational age ranged from 9 to 22 weeks. Tissue fixed in alcohol Bouin fluid and in Gendie fixative, and fresh frozen tissue were studied.

Before 12 weeks of gestational age the seminiferous tubules of the young fetal testis contain primitive germ cells recognizable by their high alkaline phosphatase and glycogen content. Acid phosphatase was the only other enzyme found in seminiferous tubules at that stage. In the interstitial tissue NADH and NADPH tetrazolium reductase and glucose-6-phosphate dehydrogenase were found in the peripheral part of the testis. $\Delta 5-3\beta$ hydroxysteroid dehydrogenase was found in trace amounts. 17β hydroxysteroid dehydrogenase was not identified.

After 12 weeks of gestation the primitive germ cells gradually disappear from the seminiferous tubules. Alkaline phosphatase activity wanes and is no longer found at 17 weeks. Glycogen disappears a little later at 19 weeks. Enzymatic activities characteristic of postnatal seminiferous tubules such as adenosine triphosphatase, 5 nucleotidase, phosphorylase, are found in low amounts. The activity of acid phosphatase increases. Interstitial tissue is abundant and harbours intense activity of enzymes engaged in energy providing metabolisms. Steroidogenic enzymes are also concentrated in the interstitial tissue. $\Delta 5-3\beta$ hydroxysteroid dehydrogenase, 17β hydroxysteroid dehydrogenase and secondary alcohol dehydrogenase.

T Taylor & W Hamilton (Glasgow) *Activity of $\Delta^5,3\beta$ hydroxysteroid dehydrogenase isomerase in the human foetal adrenal*

It is currently postulated that the activity of this critical enzyme system is probably deficient for substrates pregnenolone and DHA during the first half of gestation. This concept is here reinvestigated in an *in vitro* rate study with post incubation quantitation by a double isotope dilution technique involving group isolation of product Δ^4-3 oxosteroids according to Taylor (1).

With each foetus a series of timed incubations (max 30 min) were performed with adrenals in Krebs Ringer bicarbonate buffer containing glucose using as substrate either ^3H -pregnenolone (20 foetuses) or ^3H -DHA (18 foetuses).

Most results demonstrated conversion rates of substrate to Δ^4-3 oxosteroids in the range 0.02–0.20 mg protein/min. Tissue from a virilizing adrenal carcinoma (child 9 years) which is generally considered deficient in this enzyme system gave values of 0.80 and 0.23 conversion mg protein/min from pregnenolone and DHA respectively confirming that the foetal enzyme activity is probably low per unit mass.

However with pregnenolone adrenal glands from 3 foetuses out of 8 around 12 weeks of gestation gave high conversion rates ranging between 0.60 and 1.20 mg protein/min.

With DHA adrenals from 6 male foetuses and 2 of undetermined sex formed Δ^4-3 oxosteroids adrenals from all 6 female foetuses did not.

These results are too few for high significance but the tentative suggestion is made that a sex difference may exist in the conversion of DHA to Δ^4-3 oxosteroids. If this is so it might be postulated that an adrenal role in foetal sex development or its maintenance could be operative.

W Teller & D Beckmann (Ulm) *Studies on the influence of thyroid function on steroid metabolism in newborns*

It has been shown that newborns excrete mainly tetrahydrocortisone (THE) rather than tetrahydrocortisol (THF) (1). At this age the ratio THF/THE is less than 0.1 compared with 0.4–0.6 in older children. Hellman et al (2) as well as Gold & Cigler (3) were able to produce with thyroid hormones a shift of urinary steroid patterns towards 11 keto compounds in older individuals. In the paper to be presented the hypothesis was tested whether elevated levels of thyroid hormones (PBI) in newborns have similar influence on the preferred 11 keto pathway of steroid metabolism. Twelve apparently normal and healthy infants of both sexes were given 25 mg of propylthiouracil twice daily from the 3rd through the 9th day of life when a 24 h urine was collected for steroid analysis and blood drawn for PBI. Twelve untreated infants served as controls with urine collections and PBI determinations between the 5th and 7th day of life. The average levels of PBI were 8.7 μg per 100 ml in the untreated and 4.2 in the treated group. Steroid analyses were performed according to hydrolytic extraction and chromatographic procedures as reported previously (4). In the two experimental groups neither 11 deoxy C_{19} steroids (dehydroepiandrosterone, androsterone, etiocholanolone) nor 11 oxy C_{19} steroids (11-OH and 11-O etiocholanolone, 11-OH and 11-O androsterone) nor C_{21} steroids (tetrahydrocortisol, allo-tetrahydrocortisol, tetrahydrocortisone) showed appreciable differences. It is therefore concluded that in newborn infants the preferred 11 keto pathway of steroid metabolism is autonomous rather than influenced by thyroid hormone levels as is true in older subjects.

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4 Teller W *Z ges exp Med* 142 222 1967

R Wolter & C Delcroix (Brussels) *Serum growth hormone in normal adolescents*

Intravenous insulin tolerance tests were performed after a 12 hour fast in 60 normal pre-pubescent children and in 35 normal adolescents with all degrees of puberty. Human growth hormone was measured in serum by double antibody immuno assay. Wilhelm HGH was used as standard.

The mean curves of serum HGH response to insulin show higher values for adolescents than for children at the fasting level and during the whole test. The highest level of HGH in serum in response to insulin does not vary with age before puberty. The mean value is 17.4 ± 4.9 (S.D.) ng/ml. Half of the adolescents have higher maximal response than children with a range of 7.5 to 60.0 ng/ml. There is no difference according to sex, height or stage of puberty.

The disposal rate of unlabelled HGH (Rabensbad 22) was studied in 8 children, 4 adolescents and 4 adults. HGH was given intravenously as a single injection. A computer analysis of the disappearance curves was used to describe the experimental data by the sum of two exponential functions.

The rate of disappearance of HGH is independent of the dose given. The mean transit time of HGH ranges from 25.1 to 38.4 min in the children. It is comparable in the adolescents with a range of 29.3 to 39.9 min. Among the adults, one has a mean transit time of 29.0 min, but in the three others the range is 40.1 to 57.6 min.

According to the data presented the higher levels of serum HGH observed in adolescents, as compared with children, cannot be related to a difference in the disposal rate of HGH. It is thought therefore that the growth spurt of puberty is accompanied by an enhanced growth hormone release. Adults may have a slower disposal rate of HGH.

J C Job, P C Sizonenko & M Balage (Paris) *Étude statistique des résultats des épreuves de stimulation de la sécrétion hypophysaire d'hormone de croissance par l'insuline et l'arginine*

Z Laron, I H Hochman, M Karp A Pertzlan & L Dolberg (Petah Tikva) *Effect of gonadotrophin therapy on testicular volume and sexual development in adolescent boys with hypogonadotrophic hypogonadism*

The effect of treatment with human gonadotrophins was evaluated in 6 hypogonadotrophic adolescent boys. Treatment was initiated at 15 to 19½ years of age for periods ranging from 3 to 25 months. The dosage ranged from 1 000 to 10 000 IU/week for HCG and from 150 to 300 IU/week for HMG. An increase in testicular volume during the course of HCG alone and especially during combined HCG-HMG therapy was registered. There was also an effect on the secondary sexual characteristics but little influence on bone maturation.

Due to the slow induction of secondary sexual characteristics and the cost and inconvenience of this treatment schedule it seems inadvisable to use this therapeutic approach routinely.

Z Laron, M Karp A Pertzlan & M Burke (Petah Tikva) *The correlation between insulin growth hormone and glucose in the syndrome of pituitary dwarfism with high serum IR HGH*

Twenty eight patients with the syndrome of pituitary dwarfism and high serum immunoreactive (IR) HGH ranging in age from 6 months to 20 years were subjected to part or all of the following tests: oral glucose tolerance, arginine infusion, insulin tolerance. Determinations of blood sugar, plasma insulin and growth hormone were performed concomitantly. In addition several patients having the hereditary type of this disease underwent the same studies. For control purposes served 17 children with pituitary dwarfism due to HGH lack (hereditary and sporadic) and 20 juvenile diabetics.

It was found that both children with high (but clinically inactive) HGH and those with low HGH had low plasma insulin concentrations, which did not rise significantly during glucose infusion similar to many children with juvenile diabetes. During glucose load the response was variable. Despite a lower insulin response they had no glucose intolerance, most probably due to a metabolic "Houssay phenomenon". Interestingly, two of the parents heterozygous for the disease showed a prediabetic glucose intolerance.

lowed by an almost complete arrest of growth after an initial short growth spurt. The antibody concentration and binding capacity were extremely high already a few months after beginning GH therapy; antibodies could still be detected years after withdrawal of GH.

6 Four of these patients were related to each other.

These observations suggest that the 6 children suffer from a hereditary total GH-deficiency which is operative already before birth and which causes a lack of immune tolerance to homologous human GH.

R. Illi, A. Prader, A. Ferrandez, M. Zachmann (Zürich) *Hereditary prenatal growth hormone deficiency with increased tendency to growth hormone antibody formation ("A type" of isolated growth hormone deficiency)*

Among 19 patients with isolated growth hormone (GH) deficiency who were studied by us during the last years, 6 children could be distinguished by the following characteristic features:

1 Small body length in relation to weight at birth, less than 49 cm despite birth weights of 4000 g in two and more than 3500 g in two other children. This intrauterine growth retardation became more apparent when compared with their healthy siblings.

2 Retarded growth from birth which led to extreme dwarfism; the growth deficit expressed in standard deviations was 7.3 (± 1.0) at 2 years and 8.0 (± 0.9) at 10 years. The difference of growth deficit between these 6 children and the other patients with isolated GH deficiency is highly significant.

3 Typical face with large vaulted forehead and small nose with retracted bridge.

4 Strong anabolic action of GH during a provocative HGH therapy for 5-6 days (N retention test): 43.7% (± 12.6) N retention compared with 31.3% (± 1.5) in 6 other patients with isolated GH-deficiency ($p < 0.05$) and 15% in 21 control children.

5 Early appearance of GH antibodies fol-

A. Ferrandez, A. Prader & M. Zachmann (Zürich) *Isolated growth hormone deficiency in prepubertal children: height, weight, skinfold thickness, skeletal age and cortical thickness of bone before and under treatment with human growth hormone*

Seven patients with isolated growth hormone deficiency aged 3 to 11 years were treated with human growth hormone (HGH) for a period of 0.8 to 1.2 years. The effect on height, weight, skinfold thickness, skeletal age as well as on the cortical thickness and on the diameter of the metacarpal bones were studied.

If the changes of these parameters are expressed in standard deviations from the normal mean, the following results are obtained:

1 Before treatment height is the most retarded parameter (dwarfism). Weight is less retarded and the skinfold thickness is above the normal mean values (obesity). The retardation of the skeletal age and of the measurements on the metacarpal bones is about equal and clearly less marked than the retardation of height (no osteoporosis).

2 Under treatment there is a rapid increase in height and cortical thickness, a rapid normalization of the skinfold thickness and a moderate increase in weight indicating an augmentation of bone and muscle mass. There is also a moderate increase of the skeletal age and of the diameter of the metacarpal bones.

- 3 Gold N I & Crigler J F *J Clin Endocr* 23 156 1963
 4 Teller W *Z ges exp Med* 142 222 1967

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The ratio of the combined length of hand bones over height varied from 35–43% in 9 individuals with male hypogonadotrophic hypogonadism (HH). Between individuals this ratio appeared to be independent of bone age (range 10.7–19 years, Tanner & Whitehouse method) of height (range 137–189 cm) and of treatment with HCG or androgens. In individual cases the ratio underwent a slight rise over a number of years (maximum noted shift from 35.0 to 37.1 between ca 9.5 and 17.1 years and BA 10.2 and 15.4 years). Nine randomly selected cases of chromatin negative gonadal dysgenesis with heights ranging from 134–178 cm showed ratios ranging from 38.6–44.5%.

Bone age of HH patients in their teens and early twenties remained arrested at 15–15.5 years as long as treatment had been limited to HCG. Two untreated cases studied in their early thirties had bone ages of 18 years with open epiphyses of ulna and radius.

Methyltestosterone was the first androgen received by most of the patients. This drug had surprisingly little effect on bone age although pubic and facial hair growth were stimulated and growth in height was markedly accelerated. Thus in one case treatment for 1.2 years resulted in a height increase of 9.2 cm while bone age advanced from 15.3 to 15.4 years. Long acting mixtures of androgens (1 Sustanon and 2 Triolindren) appear to be more effective in stimulating bone maturation than methyltestosterone.

Marked growth in height and in the length of the hand bones occurred during androgen treatment at bone ages of 15–15.5 years with a rapid and steep fall in the growth rate when the bone age exceeded 16 years.

■ Weber H, Helge & E. Werner (Berlin). Pituitary tumour with hypersomatotropism precocious puberty and generalized melanosis following bilateral adrenalectomy for Cushing's syndrome.

In children Cushing's syndrome is rarely due to ACTH-dependent adrenal hyperplasia. Even

less frequently therefore a postadrenalectomy hyperplasia of the pituitary which occurs in about 10% of adult subjects under these circumstances may be observed in children.

K. K. male now 14 years old developed hypercorticism at the age of 6 years. Elevation of the already high urinary values of 17 OHC and 17 ketosteroids by metopirone and the histological finding of a diffuse non nodular adrenal hyperplasia after total adrenalectomy indicated an increased endogenous ACTH secretion and the existence of an apparently normal regulatory mechanism. Postoperatively catch up growth and a generalized cutaneous melanosis occurred. Before the age of 9 years precocious pubertal changes of the testicles secondary sex characters and the skeleton were observed. Radiographically, in 1966 an enlargement of the sella turcica was first noticed which appeared to be unilateral and increased progressively. Impairment of vision or the visual field so far did not occur.

Studies of the pituitary function revealed

1 Excessively elevated ACTH plasma levels¹ of 6 000 to 10 000 pg/ml throughout the day (normal 15 to 75 pg/ml), which however were still suppressible by high doses (8 mg/day) of dexamethasone. (The generalized pigmentation may be due to an overproduction of both ACTH and MSH).

2 Increased values of urinary gonadotrophins before the age of 9 years.

3 High plasma values of GH (40 to 80 ng/ml) following different stimuli, normal suppression by oral glucose application.

4 Normal TSH plasma values and normal thyroid function.

These results indicate a hypothalamic rather than a primary pituitary disorder to be responsible for the development of the pituitary tumour. Precocious puberty and increased GH secretion may be regarded as hormonal overlap reactions.

Courtesy of Dr. Ratchliffe, London (radio-immunoassay), Dr. Hammerstein, Berlin (bio assay) and Dr. Gnanapavan, Paris (radio-immunoassay).

P Czernichow, A H Greenberg & R M Blizzard (Baltimore) *The metabolic clearance rate (MCR) of thyrotropin*

A constant infusion technique for studying the MCR of thyrotropin was developed. In a preliminary study ^{125}I -HTSH and an immunologically pure unlabelled HTSH were injected simultaneously into 2 surgically hypophysectomized patients and serum specimens were collected over a 6 hour period. The specific activity of labelled to unlabelled hormone and the respective half-lives established that the hormones were metabolized identically. The half-life was 40 and 41 min for both patients. The volume of distribution was 19.1 and 32.1 which was 8.8 and 7.1% of total body weight. The calculated MCR was 56.2 and 53.9 l/24 hr/m.

The MCR was then studied using a constant infusion method. The mean MCR of 6 normal subjects was 34.4 l/24 hr/m² (range 24.4 to 46.1 l/24 hr/m²). Two clinically euthyroid patients with Hashimoto's disease and an elevated TSH, and one partially treated hypothyroid were in the normal range (42.4, 46.6 and 32.6 l/24 hr/m² respectively). Two patients with clinical myxedema measured 9.8 and 15.1 l/24 hr/m², well below the normal range. One patient with severe alcoholic cirrhosis and hepatic failure had a normal MCR (44.5 l/24 hr/m²) and one patient with chronic renal failure was below the normal range (19.1 l/24 hr/m²). The secretion rates were not available at the time this abstract was submitted.

The effects of dexamethasone (D) on the MCR of TSH was studied in 7 patients. Eight mg of D was administered over a period of 48 hours. In 5 subjects the MCR was measured by constant infusion before and at the end of the treatment period and in 2 after withdrawal of the drug for 30 hours. No changes were observed in any subject except for one hypothyroid who showed a small increase while on D. In addition no consistent change was observed in the thyroxine TBG or TBPA levels although a slight decrease in the free thyroxine was noted after 48 hours on D. With the pre-

vious observation of Wilbur & Utiger (*J Clin Invest* 48:2096, 1969) that D suppresses serum TSH levels and that TRF-induced TSH release is not influenced by D it was concluded that the effect of D on TSH secretion was primarily at a suprahypophyseal level.

J M Tanner, H Goldstein & R H Whitehouse (London) *Standards for height of boys and girls making allowance for parental height*

Children referred to a paediatrician for confirmation of a diagnosis of abnormally small stature not infrequently have small parents. Suppose the child is a little below the third centile of height in the regular standards, but that the parents average about the tenth centile for adults. Clearly this child is less likely to be abnormal than if the parents averaged the fiftieth centile. Some allowance should be made but it is not clear at present how much.

A chart will be presented which enables the paediatrician to read off the centile position for a child in the age range 3-9 years when proper allowance is made for parental height. Examples of its use will be given. The data from which it is constructed are the regressions of child's on parents' height for some 95 girls and 135 boys whose parents had actually been measured.

These new standards are considerably more powerful than the regular ones: that is they pick up abnormalities better. Because of the greater accuracy these standards afford it turns out that a child at the fifth centile on the regular parent unknown standards falls well below the third centile when it is known that his parents are at the fiftieth centile.

J J van der Werff ten Bosch & J J M Platkamp (Rotterdam) *Skeletal growth and maturation in male hypogonadotropic hypogonadism*

A study is being made of the growth in length of the 19 long bones of the hand, maturation of the hand and wrist bones and stature growth and the effects of treatment.

ke skeletal age 4 years) presenting the clinical features of hypsomatotropicism was studied

Basal plasma growth hormone levels were between 60 and 76 $\mu\text{U/ml}$ and rose to a maximum of 508 $\mu\text{U/ml}$ during arginine infusion and to 395 $\mu\text{U/ml}$ during insulin induced hypoglycemia. Thyroid and adrenal function were normal. Plasma growth factor activity was low. Short term treatment with human growth hormone in dosage of 0.5, 2.0 and 8.0 mg/m²/day resulted in no or minimal effect on blood glucose, plasma FFA, urinary Ca/P ratio, N/creatinine ratio and OH proline excretion. Plasma growth factor activity was minimally if at all affected by the treatment.

Contrary to the patients described by Z. Laron (1) who are supposed to produce an abnormal growth hormone molecule, this case is thought to be the first demonstration of a deficiency located at the level of the induction of plasma growth factors.

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M Menckin, J Wiebel & W Blunck (Hamburg): *Treatment of precocious puberty with chlormadinonacetate*

The effect on linear growth, bone maturation and sexual development of long term treatment with Chlormadinonacetate (CMA) in doses of 6 to 12 mg per day was observed in 9 girls (group A) with idiopathic precocious puberty and 4 patients (group B, 2 boys) with precocious puberty associated with other disorders. Mean duration of therapy was 32.8 months (range 15 to 60 months) for the two groups combined, 28.7 months for group A and 42 months for group B.

Pretreatment vaginal smears showed marked estrogenic stimulation. Prolonged therapy resulted in atrophy of the mucosa. Uterine bleeding ceased. Mean acceleration of bone age over height age of all 15 patients was 15.5 months before and 13.8 months after therapy. Respective figures were 15.2 months before and 16.3 months after treatment in group A, 15.0 months

before and 8.0 months after treatment in group B. No significant undesirable effects were observed. The difficulties of evaluating the effect of CMA on prognosis of adult height are discussed.

M David, A Ruitton & G Tell (Lyon): *Insulin secretion in eight cases of precocious puberty treated with medroxyprogesterone acetate*

Eight girls treated with Medroxyprogesterone acetate for precocious puberty (7 cases idiopathic, 1 case pineal tumour) received an oral dose of glucose (1.40 g/kg body weight). The glucosemia, insulinemia and plasma levels of NEFA were determined throughout the trial.

In 3 cases the glucosemia rise was above that of normal. In all cases insulin secretion was higher than normal. In 5 cases the peak was elevated (148, 130, 106, 110, 86 $\mu\text{U/ml}$) and early before 45 min. In 2 cases an important hyperinsulinemia was observed with a maximum peak of 440 and 263 $\mu\text{U/ml}$. In 1 case the insulin response comprised two peaks, 86 and 67 $\mu\text{U/ml}$.

In all cases the surface of the plasma insulin curve was remarkably large. The fall of NEFA at 90 min was correct, lower by 34% of the initial value in 4 cases but insufficient in 3 cases. In 1 case the NEFA did not rise again at the end of the trial.

The importance of the observed abnormalities was directly related to the duration of treatment, the dose administered and the weight gain which was observed in all patients.

In one case after 6 months of treatment the insulin response was already abnormal.

The risk and severity of such a treatment is discussed in the light of these findings.

II J Degenhart, H K A Visser & H Boon (Rotterdam): *A study of the cholesterol splitting enzyme system in normal adrenals and in adrenal lipid hyperplasia*

A severe clinical picture is seen in patients with congenital adrenal lipid hyperplasia. Almost

P H W Ruyner & B T Rudd (Birmingham)
The use of clomiphene citrate to assess pituitary gonadal function in males with delayed puberty

Clomiphene citrate, a non steroidal oestrogen analogue, is capable of inducing the release of luteinizing hormone (LH) from the pituitary in normal adult males. A preliminary assessment of the value of this response as a test of the pituitary Leydig cell axis has been performed in boys with delayed puberty or in whom abnormal pubertal development was anticipated.

The urinary and plasma testosterone to the oral administration of Clomiphene citrate (100 mg once daily for 5 days) has been measured by a competitive protein binding assay in 12 male subjects (age range 9-22 years). Measurements were continued for 2 days after the drug was stopped.

The following diagnostic categories were studied

Hypogonadotrophic hypogonadism (2 patients), Isolated pituitary GH deficiency (2), Constitutional short stature with delayed puberty (3), Prader Willi syndrome (2), Cryptorchidism (2), Penoscrotal hypospadias (1)

Basal urinary testosterone levels were below the normal pubertal range (0.4-0.6 $\mu\text{g}/24 \text{ hr}$) in all except 2 patients. Both hypogonadotrophic patients and both patients with the Prader Willi Syndrome showed no response. The 2 youngest patients aged 9 and 11 years, also showed no significant response. The remaining 6 patients showed maximal urinary testosterone levels after Clomiphene stimulation ranging from 4.7 to 36.4 $\mu\text{g}/24 \text{ hr}$ indicating a normal Pituitary-Leydig cell axis. In 3 patients the maximum response occurred 2 days after Clomiphene was discontinued. There was an increase in the maximum urinary testosterone levels recorded after Clomiphene with increasing chronological age in increasing sexual maturity.

These results demonstrate that Clomiphene citrate administration may provide a basis for a clinically useful test of Pituitary-Leydig cell axis in males with pubertal abnormality. Further

studies are required during normal puberty and of the dose and duration of Clomiphene required to obtain an optimum response.

D Schonberg (Tubingen) *Hamartoma of median eminence in precocious puberty. A new neuroradiological technique to in vivo diagnosis*

The onset of complete isosexual precocious puberty before the second year of life with neurological signs such as convulsive disorders and behavioral changes suggests brain tumour and especially hamartoma of the median eminence to be the cause. Neuroradiology is the main diagnostic tool in these cases. Although more than 40 patients with precocious puberty by hamartoma have been reported only a few detailed neuroradiological studies *in vivo* have been published (1-5). After poor results with normal fractionated pneumoencephalography a new and simple technique of directed air filling under permanent visual control was developed. Deep sedation with full cooperation of the children was achieved by Droperidol, a new neuroleptic agent. In 3 cases of early onset precocious puberty with epileptic seizures, mental retardation, hypermotility and unexplained laughing spells hamartomas of the median eminence were shown by pneumoencephalography. In 2 cases an elevated level of gonadotropin releasing factor activity supported our diagnosis.

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J L Van den Brande, M V L Du Caju, H K A Visser, W Schopman & H J Degenhart (Rotterdam) *Primary plasma growth factor deficiency (sulfation factor and thymidine factor) presenting as hyposomatotropic dwarfism*

A patient with severe dwarfism (chronological age 10½ years, height 85 cm, weight 14.0

tisol (F) and 11-deoxycortisol (S) were very low whereas deoxycorticosterone (DOC) and corticosterone (B) secretion rates were increased 7 fold. Results expressed as mg/m per day were as follows: F 1.3, S 0.023, DOC 0.35, B 1.6 (mean normal values were F 7.5, B 0.76, DOC 0.055, S 2.2). Plasma gonadotrophins were markedly increased (FSH 106 IU/L, LH 364 mIU/ml). Testicular biopsies revealed interstitial cell hyperplasia and early spermatogenesis. The karyotype was 46/XY. The pedigree showed no other affected members. At laparotomy ovaries, uterus and fallopian tubes were absent, vas deferens was incomplete, prostate was present. External genitalia consisted of small phallus, bifid scrotum, third degree hypospadias and small vagina. At puberty there was no growth of body hair or phallic enlargement. Biopsy of marked gynecomastia showed both ducts and acini. Testosterone administration produced virilization. Sexual ambiguity demonstrates strong dependence of external genitalia on androgens for male differentiation. Suppression of müllerian structures occurred despite female levels of testosterone indicating that this step in male differentiation is not testosterone-dependent. Pubertal breast development in this male supports the concept of femaleness during ontogeny unless counteracted by male hormone. Diagnosis of other adrenocortical enzymatic deficiencies is excluded by the steroidal studies. The clinical response to testosterone excludes testicular feminization. Deficiency of 17 hydroxylation must be added to the cause of male pseudohermaphroditism.

W. Zachmann, J. A. Vollmin, G. Schachenmann & A. Prader (Zurich). *11 β hydroxylase deficiency. Longitudinal steroid study in an infant.*

In a neonate girl (46 XX) with marked clitoral hypertrophy and 11 β hydroxylase deficiency was diagnosed at the age of 1 week. Without treatment the steroid excretion was followed for the first year of life.

The total 17 ketosteroids were slightly elevated.

hydroxylase deficiency could be excluded (pregnanetriol normal, pregnanetriolone absent). The total 17 hydroxycorticoids (17 OHC) were first slightly then markedly elevated. The excretion of testosterone was high. While androsterone, aetiocholanolone and dehydroepiandrosterone were detectable, no 11 keto or 11 hydroxy 17 ketosteroids were found. The excretion of tetrahydro-S (THS) was elevated already with 1 week. A gradual increase was noted up to the age of 11 months and within the following 2 months a dramatic increase took place. Tetrahydrodesoxycorticosterone (THDOC) was not detected at all up to 11 months and at 12 and 13 months was only found in small amounts. This indicates that the 11 β hydroxylation of S to cortisol is impaired while that of DOC to corticosterone appears to be intact. After administration of metyrapone the 17 OHC and THS did not further increase. Under ACTH there was a considerable increase of both 17 OHC and THS. Our results demonstrate that there are either two 11 β hydroxylating systems in the human adrenal or that one enzyme has two different receptor sites for compounds which are and are not 17 hydroxylated. In addition this case shows that even in a marked deficiency the characteristic pattern with respect to THS appeared only after 1 year. In this patient 16 α hydroxy pregnenolone (16-OH P) was detected in urine up to 6 months of age. At 13 months when the excretion of THS became very high 16-OH P was no longer detectable. It is conceivable that the physiological insufficiency of the 3 β hydroxysteroid dehydrogenase and the physiological preferential 16 α hydroxylation have prevented THS to reach high levels during the first year of life.

C. C. Forsyth (Dundee). *Adrenocortical atrophy and diffuse cerebral sclerosis. Biochemical studies.*

A boy diagnosed as having Addison's disease due to idiopathic atrophy of the adrenal glands at the age of 7 years developed the first evidence of diffuse cerebral sclerosis at 8 years.

He died at 9 years 11 months.

no synthesis of hydrocortisone, aldosterone and C_{19} compounds seems to occur in these crises, while large amounts of cholesterol and other sterols are found in the adrenals of the patients (1). A defect in the conversion of cholesterol to C_{17} and C_{19} compounds is the most likely explanation.

The enzyme system that converts cholesterol to pregnenolone has at least three activities: (i) 20 α hydroxylase, (ii) 22R-hydroxylase, and (iii) lyase (20 α R-dihydroxycholesterolisocaproaldehyde-lyase) (2).

Incubation studies with extracts prepared from acetone defatted adrenal cortex mitochondria gave the following results. No measurable conversion of labelled cholesterol into pregnenolone (<0.01%) was obtained with the post mortem tissue from a child with adrenal lipid hyperplasia (3). Yet 20 α hydroxycholesterol on the contrary, was converted into pregnenolone for about 3%. This is completely comparable with normal tissue stored at -20°C for the same period. The conclusion is that in adrenal lipid hyperplasia a defect in the cholesterol-20 α -hydroxylase is very likely. Without 20 α hydroxylation splitting of the side chain between C_{17} - C_{18} or C_{17} - C_{20} is improbable, so that neither C_{17} steroids nor androgens can be formed. This agrees with older clinical and chemical data.

appeared elsewhere (*J Clin Endocrinol* 31:162, 1970). In summary, these clearly established the specific diagnosis as 96% of the total of 1.4 mg/100 ml of plasma steroids, and 95% of the total of 103.9 mg/day of urinary steroids had 3 β hydroxy Δ^5 structure. The most interesting finding was an unequivocal elevation of urinary androsterone and 5 β pregnane 3 α ,17 α , 20 α -triol (1.4 and 1.5 mg/day respectively).

An analysis of the boy's urinary estrogens (Professor Herman Adlercreutz) has further revealed a several fold increase in estriol (19 μ g/day). All the three hormonal steroids from below the deficient enzyme step in the metabolic pathway were identified by gas liquid chromatography and gas chromatography mass spectrometry.

The patient's bile was analysed by Professor Jan Sjovall of Karolinska Institutet. He found completely normal bile acid composition qualitatively and quantitatively. As the 3 β hydroxy steroid dehydrogenase step is also necessary in the synthesis of bile acids the liver seems to have an adequate activity of this enzyme. Presumably the increased amounts of estrinol, androsterone and pregnanetriol are produced by the liver from the Δ^5 steroids dehydroepiandrosterone and pregnenetriol, which circulate in markedly elevated concentrations.

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J. Perheentupa, O. Janne & R. Vihko (Helsinki). 3 β hydroxysteroid dehydrogenase deficiency. Steroid studies in an eight year old boy.

Studies were discussed of plasma and urinary neutral steroids, urinary estrogens, and bile acids of duodenal bile in an 8 year old male first seen at the age of 1 month because of ambiguous genitalia and salt losing. The findings of his neutral steroids have already ap-

M. I. New (New York). Male pseudohermaphroditism due to 17-hydroxylase deficiency.

This is the first report of a male with 17-hydroxylase deficiency resulting in male pseudohermaphroditism with ambiguous external genitalia, absence of male secondary sexual characteristics and gynecomastia at puberty. Diagnosis was based on extensive studies of steroid metabolism including low urinary excretion of 17-ketosteroids and 17 hydroxycorticoids which did not increase after ACTH, no response of very low plasma testosterone and dehydroepiandrosterone to ACTH or chorionic gonadotropin, low urinary aldosterone and plasma renin which increased after dexamethasone. Secretion rates of 17 hydroxylated steroids cor-

ment with supplementary sodium chloride (4 g per day) the patient did better but a definite improvement was first obtained with desoxy corticosterone acetate (25 mg intramuscularly every 3 weeks)

Further studies showed a low aldosterone secretion 7 weeks old $11 \mu\text{g}/24$ hours and 10 months old below $1 \mu\text{g}/24$ hours both on supplementary sodium and after sodium restriction. Cortisol secretion was normal ($164 \text{ mg}/24$ hours) and corticosterone secretion high ($76 \text{ mg}/24$ hours). According to this the urinary C_{21} corticosteroid pattern showed normal amounts of cortisol metabolites (THE, THF, allo-THF) and increased amounts of corticosterone metabolites (THA, THB, allo-THB). Plasma levels of corticosteroids and renal and other urinary metabolites were also studied.

Besides this adrenal lesion the patient has a chronic pyelonephritis with vesico-ureteral reflux on the right side.

H. Gleispach (Innsbruck) *Results obtained by measurement of 17 KS with gas liquid chromatography (GLC) and the method of Zimmermann*

It is shown that the knowledge of the pattern of the different 17 KS is important for a correct judgement of their metabolism and a great help for a good treatment with drugs. A specific method however must be used by the determination of the 17 KS. Great differences are found between the values obtained by the photometric method of Zimmermann and the sum of the different metabolites received by GLC. These differences are greater in children than in adults and are caused by chromogens which are formed by hot hydrochloric acid hydrolysis and not received by enzymatic hydrolysis, a following solvolysis at room temperature and continuous ether extraction. The influence of these chromogens is demonstrated by the results obtained in the urines of two hirsute women where chromogens simulate high values of 17 KS. Only high values for testosterone

were found by GLC. The response to an administration with drugs is more specific by GLC measurement. The importance of the knowledge of the 17 KS pattern is demonstrated by the following example. A low excretion of 11 desoxy 17 KS increased the elevated excretion of 11 oxy 17 KS decreased in a child with AGS and salt losing syndrome when we used Florinef® as drug instead of cortisol derivatives.

H. Gleispach, H. Berger, J. Glatzl & P. Heide-mann (Innsbruck) *Puberty and the excretion of pregnanediol and pregnanetriol*

By our investigation of normal values of urinary steroids we found a high increase in the excretion of the pregnanes during puberty, the increase of pregnanetriol being greater than that of pregnanediol. This is in good correlation with our observation that the excretion of pregnanetriol increases more than that of pregnanediol under stimulation with HCG. We made the same observation stimulating 2 patients with male pseudohermaphroditism, a boy and a girl with precocious puberty and 3 boys with delayed puberty. Patients with gonadal dysgenesis showed a low base excretion of pregnanes after stimulation with HCG, the slow increase in the excretion was greater for pregnanediol than for pregnanetriol. Gonadal testosterone could not be stimulated in 2 boys with Klinefelter's syndrome with an elevated excretion of pregnanetriol and a normal one for pregnanediol, though it could be stimulated in 2 other cases of Klinefelter's syndrome with a normal base excretion of the pregnanes.

H. Knorr (München) *Determination of plasma testosterone as hexadecafluoronanoate by GLC and electron capture detection*

Two independent methods are useful for the determination of plasma testosterone. The competitive protein binding technique and gas liquid chromatography (GLC) using the electron capture detector (ECD).

The advantage of the GLC-ECD is the high specificity in low levels. Heptafluorobutyrate is

There are twelve fully documented reports in the literature of boys with the combination of adrenocortical atrophy and diffuse cerebral sclerosis. The clinical and pathological aspects of his case were outlined briefly as a background to a consideration of the detailed studies of adrenal function made during life and of the biochemistry of the brain at post mortem. During life, six 24-hour samples of urine over the period of the boy's illness were assayed for dehydroepiandrosterone, aetiocholanolone and androsterone, 11-hydroxyaetiocholanolone, 11-hydroxyandrosterone, 11-oxo aetiocholanolone, 11-oxo androsterone. There are no reports to date of such a study in this condition or in Addison's disease. In addition, assays were made of tetrahydrocortisol, allotetrahydrocortisol, tetrahydrocortisone, tetrahydrocorticosterone, allotetrahydrocorticosterone and tetrahydro-11-dehydrocorticosterone. These steroids have been examined once in this syndrome by Dr M. Dirmkjaer Nielsen of Copenhagen and they have been examined in Addison's disease by Dr Nielsen and by Dr W. S. Cost of The Netherlands. The findings in this boy were compared with their work. The brain biochemistry was investigated by Professor J. N. Cumings of the Department of Chemical Pathology, The National Hospital, London and shed further light on the nature of the syndrome which is a specific entity. The two likely theories of causation involve either an error of metabolism common to the adrenal cortex and the brain, or the possibility that both the adrenal and brain pathology are due to an auto-immune disorder. Evidence for these theories was presented and reference was made to the likely mode of inheritance of the condition.

J. Girard, H. R. Hirt, U. Buhler & M. Vest (Basel). *Antibodies to the biologically active amino acid sequence of corticotrophin in an infant treated with synthetic β^{1-25} corticotrophin*

An infant treated with synthetic β^{1-4} corticotrophin (Synacthen, CIBA) developed anti-

bodies against the biologically active part of the ACTH molecule. Subsequently the sera of 5 other children—for different reasons on long term ACTH treatment with Synacthen—were investigated, and antibodies detected in 2 of these 5 patients. With a radioimmunological method the binding of ACTH to the patients' sera was identified as an antigen-antibody reaction. All sera were found to have a high avidity and a binding capacity of over 1 μ g of corticotrophin or Synacthen respectively per ml of serum. The possible danger of inducing antibodies against the biologically active part of the ACTH molecule by long term treatment requires a careful control of steroidogenesis and the sera should periodically be checked for antibodies.

K. H. Kristensen, K. E. Petersen, E. Thomsen & N. C. Hansen (Copenhagen). *Selective hypoadosteronism. Report of a case in an infant with determinations of secretion rate for aldosterone, cortisol and corticosterone*

The patient, a boy with a birthweight of 3350 g, was born by normal delivery after a normal pregnancy. The parents were young and healthy, but their first child, a boy, died suddenly 5 weeks old with the same clinical symptom as the patient.

The patient was seen in the hospital when 3 weeks old with poor thriving but no vomiting at 20 days old the weight was 130 g below birthweight. On admission the findings were: Signs of weight loss, dehydration, hyponatremia (serum Na 115 mEq/l), hyperkalemia (serum K 7.0 mEq/l) and acidosis (serum HCO_3^- 15.9 mEq/l). External genitalia had a little more pigmentation than usual in Scandinavian infants, the penis seemed to be enlarged (50 \times 12 mm).

Urinary 17-ketosteroid excretion was normal (0.1–0.2 mg/24 hours) and it was not possible to detect pregnanetriol in the urine. Congenital adrenal hyperplasia therefore seems excluded but the salt loss could be due to hypoadosteronism as first described by Visser. On treat-

was developed. In this an aliquot of a 24 hour urine collection was subjected to hot hydrolysis at neutral pH for 6 hours (Fotherby 1959). Liberated steroids were extracted with diethyl ether and the ketones were separated from the non ketonic compounds by Girard reaction. DHA was separated by paper chromatography in Bush A system and after eluting the concentration of DHA was measured spectrophotometrically by Zimmermann reaction (James & De Jong 1961). The overall average recovery of the procedure was 85% where DHA 7 α -T(n) sulphate potassium salt added to the urine and taken all through the analysis. The identity of DHA was confirmed after making various chemical derivatives and checking up their chromatographic mobilities against derivatives prepared from authentic steroids.

When this procedure was applied to the urine from 10 children aged 9-15 years with various adrenocortical disorders the levels of DHA were found to be above normal. Of these 10 patients 2 had adenoma, 4 had carcinoma and 4 had hyperplasia, the value of DHA was between 2.8 and 5.1 mg/24 hr (mean 3.6 mg/24 hr). All these patients had to undergo surgery and in each case DHA was estimated after 1 month and the value of DHA was found to be between 0.38 and 1.1 mg/24 hr (mean 0.69 mg/24 hr).

From these results it is possible to conclude that urinary DHA determination could be used for the diagnosis of adrenocortical disorders in children.

E. Gray & W. Hamilton (Glasgow) *Excretion of dehydroepiandrosterone in cystic fibrosis*

The abnormality of the mesonephric structures as described in patients with cystic fibrosis (CF) by Kaplan et al. (2) suggests that the intrauterine stimulus for their development might have been abnormal. This has led to a consideration of dehydroepiandrosterone (DHA) and its ester dehydroepiandrosterone sulphate (DHAS) in patients since DHA as a precursor

Patient Age Sex	Clinical rating	17 OX (mg/24 hr)	DHAS (μ g/24 hr)	Normal	
				DHAS	DHAG
18 ♂	Severe	2.5	30	392	65
20 ♂	Good	4.7	187		
8 ♂	Good	0.28	46.9	42.7	54.8
10.5 ♂	Severe	0.12	5.6		
7 ♂	Severe	0.44	13.6	6.29	4.96
7 ♂	Good	0.6	13.5		

These results could indicate a reduced synthesis of DHAS by the adrenal cortex, a reduced release of formed DHAS from the adrenal or defective resulphation of free DHA at the hepatic and/or intestinal levels.

of testosterone is important in the genital development of the male foetus.

DHAS and dehydroepiandrosterone glucuronide (DHAG) were extracted from urine of patients according to the method of Fotherby (1) and Kellie & Wade (3) respectively. Quantitation was by electron capture detection of DHA heptafluorobutyrate from a 9 foot hybrid column (7 ft 1° NGS 2 ft 1° SE 30) against standard testosterone heptafluorobutyrate.

Our studies of CF patients rated clinically as severe shows low DHAS levels compared to normal values reported by Loras et al. (4). In all cases total 17 oxosteroids (17 OX) excreted daily are less than normal.

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A. J. Hadjian, M. Cheddim & E. M. Chambaz (La Tronche) *Study of cortisol binding proteins by a competition technique and different graphical calculation methods. Application to the neonatal period*

A competitive protein binding technique was used in order to study the characteristics of

the most common derivative of steroids for ECD-GLC

Kirschner (1969) described a new derivative, hexadecafluorononanoate, which is more than twice as sensitive in the ECD as heptafluorobutyrate. The method includes the following steps: Ether extraction of 10 ml plasma; I Thin layer chromatography; Derivation; formation II Thin layer chromatography; Gas chromatography with ECD.

Correction for losses by a ^3H testosterone internal standard.

Using this derivative we determined the plasma testosterone level in boys before and during the puberty. Testosterone is excreted in urine also before the puberty deriving from adrenal androstenedione. Using the ECD-GLC technique testosterone was not detectable in plasma of prepubertal boys. Normal values of plasma testosterone in childhood and in puberty will be presented.

B. T. Rudd & B. Morris (Birmingham) *Methodological difficulties in the measurement of C_{19} steroids by competitive protein binding (CPB)*

The asymptotic form of standard curves prepared from 3rd trimester pregnancy plasma is present over a wide range of dilutions and cannot be eliminated by dilution alone or isolation and use of the β globulin fraction with a high affinity for 17β ol steroids. Adequate curves can be obtained when the total number of binding sites is low, as is demonstrable with plasma obtained 10 days post partum diluted 1 in 50. All plasma should be titrated for binding sites before deciding which is the most sensitive curve obtainable. Highly diluted pregnancy plasma (1:300) does not keep at 4°C and yields curves with decreased binding affinity after 24–82 hours. Prefrozen (-20°C) aliquots of undiluted plasma keep well without change in binding affinity. Inadequate mixing of labelled plasma with solid phases to separate free from protein bound steroid, leads to flat curves. Exposure time to solid phase is critically marked

variation in time between paired analyses gives curves of poor precision.

A major source of error is that due to an increase in method blanks from sources other than the biological material. These are common merceril binders in TLC plates, solvents used for elution e.g. ethanol, methanol, ether which give values of apparent testosterone of 0.2–2.0 ng. Every solvent used should be tested for quality in the assay, and plates pre-washed. Adequate TLC systems must be used to separate diols from testosterone, as they compete avidly for binding sites. Enzymatic conversion of Δ^4 androstenedione to testosterone before assay requires rigid control of quality including $(\text{NH}_4)_2\text{SO}_4$ precipitation and acetone extraction as the enzyme itself contributes significantly to blank of the method.

D. Gupta, K. Rager & R. Huenges (Tubingen) *Studies on testosterone protein reactions in children in relation to age, sex and different endocrinopathies*

The binding affinity (BA) and binding index (BI) of plasma proteins for testosterone were studied by a technique based on the principle of precipitation by dextran coated charcoal. The binding parameters were examined in relation to concentration of both proteins and steroids. Some of the factors that influence the binding capacity of the specific testosterone binding globulin were also investigated.

Testosterone binding affinity levels were determined in children before and during puberty in relation to their sex and maturation. Several other groups of children with various endocrine disorders such as precocious puberty, adrenogenital syndrome, hypopituitarism, hypogonadism and children receiving cyproterone were also studied.

S. B. Pal & W. Teller (Ulm) *Urinary excretion of dehydroepiandrosterone in children with adrenocortical disorders*

A systematic analytical procedure for the determination of urinary dehydroepiandrosterone

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Using this derivative we determined the plasma testosterone level in boys before and during the puberty. Testosterone is excreted in urine also before the puberty deriving from adrenal androstenedione. Using the ECD-GLC technique testosterone was not detectable in plasma of prepubertal boys. Normal values of plasma testosterone in childhood and in puberty will be presented.

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S. B. Pal & W. Teller (Ulm) *Urinary excretion of dehydroepiandrosterone in children with adrenocortical disorders*

A systematic analytical procedure for the determination of urinary dehydroepiandrosterone

ification of urinary steroids. The high resolution on this type of column saves complicated purification steps. The steroids are chromatographed as trimethylsilyl ether or if an adrenocortical side chain (20-one 17 α or 20-one 17 α 21-diol) is present as methoxime trimethylsilyl ether derivatives. For the identification of unknown urinary steroids the same columns are used in a combination of gas chromatograph and mass spectrometer. With a new technique of splitless injection quantitative determination of urinary steroids are also possible. The usefulness of this technique for quantitative analysis is illustrated by the example of THS and THDOC. In 16 children (0.1–14 years old) the excretion of THS under

basal conditions ranged from <5 to 60 $\mu\text{g}/24$ hr and in 18 adults (16–36 years old) from 5 to 76 $\mu\text{g}/24$ hr whereas THDOC could not be detected. After administration of metyrapone 16 normal adults excreted 990–8700 $\mu\text{g}/24$ hr of THS and 120–1100 $\mu\text{g}/24$ hr of THDOC.

An untreated girl (1.1 years old) with congenital adrenal hyperplasia due to an 11 β -hydroxylase deficiency excreted 2800 $\mu\text{g}/24$ hr of THS and 32 $\mu\text{g}/24$ hr of THDOC. Three untreated patients with congenital adrenal hyperplasia due to a 21-hydroxylase deficiency showed a slightly elevated THS excretion (90–154 $\mu\text{g}/24$ hr).

C. G. Bergstrand

cortisol binding proteins in human plasma. After equilibrium, charcoal coated dextran was used to separate the free (F) from the bound (B) steroid. From the values obtained for B/F and total cortisol, different graphical representations were constructed for the identification of the binding species present in plasma and the calculation of the binding parameters for transcortin. The Scatchard plot appears the simplest approach and yields satisfactory results for clinical research purposes as compared with more elaborate calculation methods (e.g. Baulieu E. E. & Raynaud J. P. *Europ J Biochem*, 13: 293, 1970).

The values obtained for CBG in adult and pregnancy plasma are in agreement with the values found in the literature (6 to 8×10^{-7} M for the binding capacity and 4 to 7×10^{-8} l/M for the affinity constant K_t at 3°C). With this type of technique, the temperature appears to be the most critical point as it greatly influences the K_t value.

The CBG characteristics obtained by this approach in infant and newborn plasma are presented. The affinity constant K_t was found similar to the adult values, whereas the binding capacity was always low ($\leq 3.5 \times 10^{-7}$ M). These low values were corrected to the adult levels within the first 3 or 4 weeks of life. Low values were also found in the cord plasma as compared with the average adult binding capacity, this is in agreement with the previous finding by De Moor's group using gel filtration (De Moor et al. *J Clin Invest* 41: 816, 1962).

Some technical difficulties encountered in the accurate measurement of cortisol basal level and subsequent binding studies in the newborn and cord plasma are discussed. In such plasmas containing high level of interfering compounds treatment with an adsorbent before the binding studies was found very useful.

This type of approach results in a simple and relatively rapid method which may facilitate the study of various biological fluids. Its results compared well with those obtained using equilibrium dialysis which may be considered as the reference method. On the other hand, more

information is obtained than with the simple gel filtration technique.

At the same time measurement of plasma cortisol concentration by CPB using the patient plasma as the source of the binding protein might be carried out as described by Pegg & Keane (Pegg M. J. & Keane, P. M. *Steroids*, 14: 705, 1969).

R. Rappaport (Paris) *A technique of dosage of cortisol by competitive protein binding*

The technique of cortisol estimation by competitive protein binding analysis has been applied to measurements in plasma. 0.2 ml plasma volumes were used for micromethod, 0.07 ml for the ultra micromethod. A careful control of the temperature during incubation, shaking and centrifugation allowed excellent reproducibility. The temperature should not exceed 10°C at any moment, in order to obtain maximum binding of the steroid on the protein. Separation was achieved by Fuller's earth (20 mg). The precision of the method was evaluated by determining the coefficient of variation between 5.1% and 8.1% for the micromethod. Known amounts of cortisol were added to a cortisol free plasma with a recovery close to 100%. Normal values for men were 11.7 ± 2.5 $\mu\text{g}/100$ ml by the micromethod and 13.4 ± 3.1 $\mu\text{g}/100$ ml by the ultramicro method ($r=0.82$, $p<0.0001$). For both methods we used human plasma at 2.5% and 0.5% dilution. The standard error is 0.5 for a plasma concentration 6.3 $\mu\text{g}/100$ ml. Use of the method is limited by its low specificity. The ^3H cortisol displacement from the Fuller's earth was measured for 11 deoxycortisol, corticosterone, 11 desoxycorticosterone, testosterone, prednisone and prednisolone. Practical application for clinical investigation are discussed.

J. A. Vollman (Zurich) *Separation and identification of urinary steroids on glass capillary columns in a combination gas chromatograph-mass spectrometer*

Glass capillary columns have been used for the gas chromatographic separation and iden-

VIRUS SEROLOGY AND SERUM IgE LEVELS IN CHILDREN WITH ASTHMATOID BRONCHITIS

T FOUCARD T BERG S G O JOHANSSON and B WAHREN

From the Department of Paediatrics and the Blood Centre University Hospital Uppsala
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The relationship between asthma and bronchitis with signs of obstruction (wheezing) has been discussed for many years. Among children who react to respiratory tract infections with wheezing there is an overrepresentation of children who will become asthmatics. Several retrospective studies (10-12, 14, 29, 30, 35, 39, 42) of such children and a few prospective studies (19, 44) have shown an asthma frequency varying from about 5 to 50%. These figures can be compared with the asthma frequency of about 1% among Swedish school children (26) and of about 5% among American college students (16).

In children with wheezing during respiratory tract infections it is often difficult in the single case to give a correct prognosis as to the risk of developing asthma. Recurring symptoms, higher age of onset, allergic heredity and previous atopic manifestations are unfavourable prognostic factors (5, 12, 14, 31).

In 60% of adults and older children with extrinsic asthma Berg & Johansson (3, 20) have found a pathological increase in the serum level of immunoglobulin E. A study of the IgE levels in infants with astmatoid bronchitis might therefore be of value in assessing the prognosis.

During the last decade studies have shown that infections giving rise to asthma like symptoms in children are predominantly of viral aetiology (1, 6, 28, 37). Bacteria play a far

more inferior role at least in the early stage. (1) Of approximately 150 known airway viral pathogens there are only a few causing most of the infections accompanied by symptoms of bronchial obstruction. The most common of these viruses are the respiratory syncytial (RS) virus and the parainfluenza viruses (6, 28, 37).

Whether the obstructive symptoms caused by RS virus infections are of a non specific mechanical nature (37) or have an immunological background (8, 15) is still not established.

The aim of this study has been to investigate the serum IgE levels in a group of children with astmatoid bronchitis and relate these levels to the virus serology and other factors of prognostic interest.

MATERIAL AND METHODS

Definitions

Astmatoid bronchitis is defined as a condition with dyspnoea and expiratory wheezing in children with signs of respiratory infection. *Bronchiolitis* in this work and as commonly done in Scandinavia is defined as a more severe clinical picture occurring especially in infants less than 6 months old accompanied by pronounced dyspnoea, tachypnoea, sometimes cyanosis, hyperinflation of the lungs and marked suprasternal and subcostal retraction. Most of these infants have expiratory wheezing too. Cases bordering bronchiolitis and bronchitis are not uncommon and may be difficult to evaluate. In the Anglo Saxon literature the term bronchiolitis is sometimes used in a broad meaning including our term astmatoid bronchitis. Some authors have used an

BOOK REVIEWS

J Huter & M Zehntbauer *Übergang von Medikamenten in die Muttermilch und Nebenwirkungen beim gestillten Kind* Georg Thieme Verlag Stuttgart 1970 80 pp DM 22.—

In literature there are very few monographs on the transfer of drugs from mother to infant via breast milk and on their secondary effects. Therefore this book giving a systematical and comprehensive survey on this subject is welcome. About two hundred of the more common and important drugs are discussed and for each drug the results of studies on animals or on man are briefly described. The chapters dealing with the most common drugs end with a short clinical conclusion. For further studies the reader is referred to 346 references.

The book is of value in pediatric (neonatal) as well as in obstetric practice.

Tor Landberg

H G Schwarzacher & U Wolf (eds) *Methoden in der medizinischen Cyto-genetik* Springer Verlag Berlin Heidelberg and New York 1970 186 pp illus DM 48.—

Clinical cytogenetics is a rapidly expanding branch of medical science. Over the past 10 years it has developed into an important aid in the diagnosis of birth defects of various kinds. It is also of interest for the oncologist. Early research in this field employed complicated methods which called for the co-operation of a tissue culture department. The introduction of the blood culture method in 1960 made routine analysis of human chromosomes available also to less specialized laboratories and this is still the main method for clinical cytogenetics. The presence of mosaicism for instance sometimes necessitates the analysis of other tissues too. Autoradiographic analysis of DNA synthesis patterns in individual chromosomes often gives valuable information on the identification of abnormalities. During recent years important studies of meiosis in man have been made and such studies are of increasing importance for the clinical analysis of the germ line of adult men and often help to clarify for instance translocations. All these aspects are admirably covered by different authors in this book, mainly from a methodological point of view. The book is very comprehensive and well illustrated. It discusses various technical modifications and gives detailed schedules for the use of these sometimes involved methods.

It should prove a very valuable manual for all engaged on cytogenetic research although no doubt many laboratories use further modifications and special techniques. The rapidity of expansion in this field is illustrated by the lack of references to methods for prenatal chromosomal analysis using amniotic fluid cells and to methods for the identification of Y chromosomes in interphase nuclei.

Bengt Ållen

O Oetliker (ed) *Nephrologie im Kindesalter I Pädiatrische Fortbildungskurse für die Praxis* Vol 27 Karger Basel and New York 1970 117 pp US \$7 20

R Frey & Th Baumann (eds) *Nephrologie im Kindesalter II Pädiatrische Fortbildungskurse für die Praxis* Vol 28 Karger Basel and New York 1970 114 pp US \$7 20

The current volumes of this series are dealing with kidney diseases in childhood, mainly the glomerulopathias and urinary tract infection.

The books report papers from the annual congress of Schweizerischen Gesellschaft für Pädiatrie giving both theoretical and practical aspects of the mentioned groups of diseases. The value of needle biopsy of the kidney is briefly outlined by J Ehrensperger, Lausanne, and the pathological changes in the glomeruli and their clinical significance more extensively discussed by E Habib, Paris. Experimental immunological lesions to the glomeruli as well as renal engagement in collagen diseases are questions elucidated by other authors. Pylonephritis and malformations of the urinary tract, diagnostic problems in urinary tract infection and post-operative treatment of pyelonephritis in urinary tract infection are subjects treated. In other papers is dealt with basic renal physiology, radiologic findings in childhood urinary tract disease and tubular dysfunction.

P Royer, Paris, contributes with an analysis of the renal hypertension in children and a valuable survey of renal disease in the newborn and in early infancy.

In those two small books in all 16 papers are presented, 6 in French and 10 in German. There are no multilingual summaries. Most papers are informative although short and the books give a rapid and actual orientation of the subjects mentioned.

Sture Sjöblad

with the CF technique. In addition to these children another two children 9 and 15 months old showed the same titer rise against RS virus but these cases have been recorded only as ECHO virus infections since at least a 10 fold titer rise was found against ECHO 3 and 7 respectively.

For the cases recorded below the zero line as possible parainfluenza virus infections the titer changes were of another type. In the first serum sample they had titers ranging from $1/20$ to $1/160$ but in the second sample the titers had decreased in almost all cases. In the third serum sample the titers had decreased significantly compared to the first sample in all analyzed cases. Treatment of these children's acute sera with dimercaptoethanol gave no reduction of the titers indicating that the antibodies were not of IgM nature.

Almost all RS virus infections occurred in infants younger than 15 months of age. In this age group significant titer rises against RS virus were demonstrated in slightly more than 50% of the infections where a virus etiology could be shown. For infants who were less than 9 months of age the corresponding figure was 75%. Significant titer rises to adenovirus were found in sera from only 3 children who were 21-33 months old. Two of these children also showed a significant titer rise against another virus.

RS virus and parainfluenza virus infections showed an uneven distribution during the investigation period (Fig. 2). Parainfluenza virus infections (type 3) were common during the

Table 1 Virus serology findings in 72 infants with asthmatoïd bronchitis. 'Possible infections' means infants with titer rises less than 4 fold for RS virus or high initial titers for any virus.

Virus	No of cases with > 4 fold titer rise	Additional cases with possible infections	Negative serology
RS	13	2	
Parainfluenza	5	12	
Type 2	1	2	
Type 3	4	10	
ECHO	13		
Type 3	3		
Type 6	2		
Type 7	1		
Type 9	4		
Type 11	3		
Adeno	3	1	
Influenza A	1		
	35 (48%)	15 (21%)	22 (31%)

first winter. The first RS virus infections were noted in May-June 1969 and during the following autumn and winter this virus was found to be the dominant infective agent.

In two cases a simultaneous titer rise was found against two different viruses: in one case RS virus + adenovirus and in the other ECHO virus + adenovirus.

Table 1 summarizes the serologically demonstrated virus infections. RS virus infections were most often demonstrated (18%). The total number of ECHO virus infections was as high but these infections were distributed among 5 different types.

Swabs from nose and throat for routine bacterial culture (47 children) showed a growth of potential airway pathogens in 14 cases. Of these 1 had *Hemophilus influenzae*, 4 had *Hemophilus parainfluenzae*, 2 had *β hemolytic streptococcus*, 1 *Klebsiella* and 6 *Staphylococcus aureus*. Three of these 14 children showed significant titer rises against RS virus or adenovirus.

The clinical pictures, white blood cell counts and sedimentation rates were rather similar for the different kinds of virus infections. The RS virus infected infants had fever ($\geq 38^\circ\text{C}$) more often than other virus infected infants.

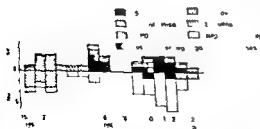


Fig. 2 Distribution of virus infections during the investigation period (serological diagnosis) in 72 infants with asthmatoïd bronchitis. Symbols as in Fig. 1.

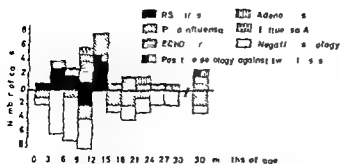


Fig 1 Virus serology in 72 infants with asthmatic bronchitis. Symbols above the zero line indicate at least 4 fold titer rises. Filled symbols below the zero line indicate titer rises less than 4 fold for RS virus or high initial titers for any virus.

upper age limit of two years (10/12/44) others have not (19/37/42). Atopic manifestations include hay fever, extrinsic asthma, certain cases of eczema and occasional crises of urticaria.

The series consists of all but two children with asthmatic bronchitis hospitalized for a few days at the Department of Pediatrics, University Hospital Uppsala during the period November 1968 to March 1970 (49 children). Besides 23 children treated as outpatients during the same time are included. In total there were 46 boys and 26 girls. Forty-four of the children (aged 2-33 months) had their symptoms for the first time while 28 children (aged 5-66 months) had had one or more similar attacks earlier. These former attacks had all occurred in connection with respiratory infections according to the parents. All the patients were seen by one of the authors (T.F.).

In the hospitalized children the blood samples were obtained by venipuncture during the first 24 hours. In a few cases the sampling was delayed until 48 hours. A second sample was taken from all patients 2-4 weeks later (mean 20 days) and a third sample another 1-4 months later. After centrifugation of the clotted specimens the sera were withdrawn and kept frozen at -20°C until analyzed.

White blood counts, total eosinophil counts and sedimentation rates were done in the acute phase in all but a few of the outpatients. Some additional investigations were performed on the hospitalized children: nose and throat swabs for routine bacterial culture, smears of bronchial or nasal secretions for examination of eosinophils and pulmonary X rays.

Immunoglobulins

Quantitative determinations of the immunoglobulins G, A, M and D were done on the sera of the first 30 patients by single radial immunodiffusion in agar gel according to Mancini et al. with minor modifications (23). The IgE concentrations were measured by the radioimmunosorbent technique (RIST) of Wide & Porath (40) as applied for IgE determination by Johansson et al. (21). The mean error of the method calculated as the standard deviation (SD) in duplicate determinations was 14%. The IgE concentrations

obtained were expressed as percent of the arithmetic mean value for healthy children of the same age according to Berg & Johansson (2/22).

Reduction with 2-mercaptoethanol

0.4 ml of serum diluted 1/4 was mixed with 0.05 ml of 0.2 M 2-mercaptoethanol and incubated for 1 hr at 37°C . 0.05 ml of 0.4 M iodoacetamide was added to prevent re-aggregation (13). The supernatant was used for assay.

Complement fixing (CF) antibodies

The following antigens were used for complement fixation: influenza viruses A/2 and B/RS virus, adenovirus type 6, parainfluenzaviruses types 1/2 and 3 all prepared by Dr M. Grandien, National Bacteriological Laboratory, Stockholm. Serum titrations were made in doubling dilutions starting with 1/5 by the microtiter method (36). A 4 fold titer increase or more was considered significant. Patients with titers >40 in the acute phase or significant titer decreases between the first and the second sample were classified as possible infection as were titer rises less than 4 fold for RS virus.

Neutralizing (NT) antibodies

ECHO virus types 3/4/6/7/9 and 11 were used for neutralization with patients' sera in semi-solid agar cultures of green monkey kidney (GMK) cells (27). A >10 fold titer increase was considered significant; this corresponds to a >4 fold titer increase in conventional neutralization tests.

Virus isolation

Due to inadequate facilities for isolation of RS virus and parainfluenza virus these tests were omitted after a preliminary trial.

RESULTS

The age distribution of the children is shown in Fig 1. Sixty-one percent (27 patients) of the children wheezing for the first time were less than 1 year old and 89% (39 patients) were less than 2 years old. The corresponding figures for the children who had had similar symptoms one or more times before were 36% (10 patients) and 75% (21 patients) respectively. The ages of the six children who were older than 30 months were 33-66 months. Of these children all except one (33 months old) had experienced wheezing before.

The results of the virus serology studies may be seen in Fig 1. The two cases recorded below the zero line as possible RS virus infections showed a titer rise from $<1/5$ to $1/5-1/10$.

Atopic manifestations were noted in the past or present in 43% (9 of 21 cases) of parents and/or siblings among the children with high IgE while the corresponding frequency was 31% (16 of 51 cases) among the children with normal IgE. Only 3 children had had atopic eczema before. Two of these children were recidivating wheezers and had IgE levels of 44% and 88% of the mean for age. The third child was a first time wheezer with an IgE level of 174% which did not exceed 2 S.D. of the mean for age.

Blood eosinophilia ($>400/\text{mm}^3$) was noted in 67% (14/21) of cases with high IgE compared with 2% (13/51) of cases with normal IgE ($p < 0.002$). In most of the children with eosinophilia the number of eosinophils was low or normal in the acute phase and high in the convalescent phase. In the group with high IgE eosinophilia was about as common among first time wheezers as among recidivating wheezers. In the group with normal IgE however eosinophilia was more common among the recidivating wheezers but the difference was not significant. More than 10 eosinophils in secretions was noted in only 2 of 48 children investigated in the acute phase. Bronchial secretions were examined in one of the infants and nasal secretions in the other. Both infants aged 7 and 11 months respectively were first time wheezers and had a normal blood eosinophil count. The IgE levels were 369 and 454 respectively of the mean for age. At the second examination 4 weeks later the total eosinophil counts were $950/\text{mm}^3$ and $733/\text{mm}^3$ respectively. The IgE levels had then decreased to 329% and 406%.

The age of onset of the first symptoms showed no significant difference between children with high and those with normal levels of IgE.

DISCUSSION

The age distribution shows rather few infants in the age group of 0-6 months compared with other studies (12, 17, 37). This may depend on the fact that a real RS virus epidemic was not seen during the investigation period.

The sex distribution in our study with 64% boys and 36% girls is in agreement with reported experiences of asthmatoïd bronchitis (5, 12, 29, 35). The same sex distribution is also typical for bronchial asthma.

Several studies have shown the RS virus to be the most common virus in bronchiolitis and bronchopneumonia in infants (6, 28, 37, 43). Frequencies between 15% and 75% have been noted in bronchiolitis and between 10% and 35% in bronchopneumonia. During some epidemics even higher frequencies have been found (9, 33, 38). In our study 24% of the children showed titer rises against the RS virus but only $2/4$ of these titer rises were 4-fold or more. However Ross et al (32) state that it may take 4-6 weeks especially in younger infants before maximal serological response is achieved. In this study the mean period of time between the first and the second sample was 20 days. It is therefore possible that some of the cases with a 2 fold titer rise from the first to the second sample might have risen to a significant level if the second sample had been taken later. In the third serum sample from these cases the titers were again as low as in the first sample.

Significant titer rises to parainfluenza virus were found in only 5 children. Four had titer rises to type 3 and one to type 2. Parainfluenza viruses have certain antigens in common explaining why cross reactions may be seen between the different types (7). In our study no heterotypic antibody response was found but a definitive diagnosis requires virus isolation. In addition to these cases titers ($1/100$) against type 3 were demonstrated in 11 acute sera indicating that this virus was prevalent in the population during that period. The relationship between the children's wheezing and a parainfluenza infection must in these cases be regarded as uncertain but cannot be excluded. Both Schumacher (34) and Chanock et al (7) have reported parainfluenza virus infections with a late onset of wheezing. These studies suggest the possibility that some of our children with high initial titers had experienced a recent

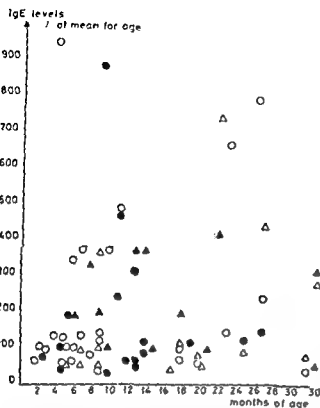


Fig. 3 Relationship between virus serology findings and serum IgE levels in 72 infants with asthmatoïd bronchitis. ○ First time wheezers negative virus serology ● first time wheezers positive virus serology △ recidivating wheezers negative virus serology ▲, recidivating wheezers positive virus serology

but this difference was not pronounced. Only 1 of the children with RS virus infection was severely ill.

Pulmonary infiltrates were noted in 12 cases of which 4 had significant titer rises against RS virus, 2 against parainfluenza virus type 3 and one against ECHO virus type 9.

Determinations of immunoglobulins G, A, M and D in the first 30 consecutive patients yielded grossly normal results. In only 7 cases a moderate rise of immunoglobulin G was noted and in 4 cases a moderate rise of immunoglobulin A. The immunoglobulin M levels did not in any case exceed 2 SD of the mean for age. Because of these results no further determinations of these immunoglobulins were made.

Determinations of immunoglobulin E showed high levels (> 2 SD), corresponding to 232–933% of the mean for age, in 21 cases. Eleven of these children had wheezing for the first time (mean IgE 495% in acute phase, 496% in convalescent phase) while the other 10 children had had one or more similar attacks earlier (mean IgE 415% and 378% respectively). Eleven of the children had a higher IgE level in the first sample, the other 10 children in the second. In 6 of the children the IgE level exceeded 2 SD of the mean for age only in the acute or in the convalescent serum.

Positive virus serology was found to be as common among the children with high IgE as among those with normal IgE (Fig. 3). Also RS virus infections were evenly distributed between children with high and normal IgE. However the RS virus infected children wheezing for the first time had all normal IgE levels while 4 out of 5 of the RS virus infected children with former asthmatoïd symptoms had high IgE levels (Table 2).

Table 2 Asthmatoïd bronchitis. Relationship between immunoglobulin E levels, virus serology and some clinical and laboratory findings prevalent in bronchial asthma. The IgE levels are related to the upper 2 SD of the arithmetic mean value for age.

IgE levels	Children wheezing for the first time		Children with recidivating wheezing	
	> 2 SD	< 2 SD	> 2 SD	< 2 SD
Number of cases	11	33	10	18
Virus serology positive				
Total	3	14	6	11
RS	—	8	4	1
Allergic heredity in parents and/or siblings	5	12	4	4
Eosinophils $\geq 400/\text{mm}^3$	7	6	7	7
Other atopic manifestations	—	1	2	—
Mean age of onset (months)	13.4	11.2	12.4	8.7

Atopic manifestations were noted in the past or present in 43 (9 of 21 cases) of parents and/or siblings among the children with high IgE while the corresponding frequency was 31 (16 of 51 cases) among the children with normal IgE. Only 3 children had had atopic eczema before. Two of these children were recidivating wheezers and had IgE levels of 444° and 885° of the mean for age. The third child was a first time wheezer with an IgE level of 174° which did not exceed 2 SD of the mean for age.

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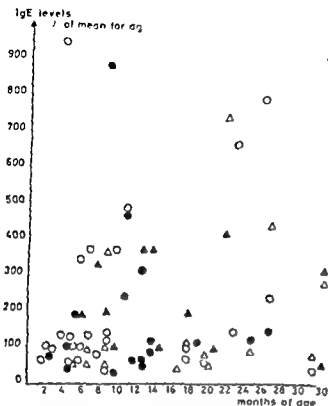


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Children with high IgE levels had a higher frequency of allergic heredity, eosinophilia in blood or secretions and previous eczema. They were also older when they had their first wheezing episode. However, the differences were rarely statistically significant. The same differences between children with high and normal IgE levels were found whether they were first time wheezers or not.

To carry out allergological investigations in a conventional way with intracutaneous and provocation tests is very difficult in infants and small children. The possibility of an *in vitro* diagnosis of atopic diseases would therefore be of great interest. In spite of the limitations discussed above, our impression is that the serum IgE level can be of help in assessing the possible atopic state of children with wheezing related to respiratory tract infections. In conditions such as asthmatoïd bronchitis with an eventual allergic component and where the possible allergens are unknown, estimations of the serum IgE level is probably the best immunological screening method. A high value should call for further allergological investigations. A normal value on the other hand should lead to further investigations when there is a clinical suspicion of atopy. The sera may then be tested *in vitro* for reaginic activity against common allergens in childhood (41). This procedure will probably facilitate further allergological investigations (4).

We intend to make repeated controls for some further years of the children in this study including a follow up of clinical histories, determination of serum IgE levels and possible reaginic antibodies against some of the most common allergens. In this way we hope to obtain a better understanding of the value of these immunological *in vitro* methods as diagnostic and prognostic adjuncts in asthmatoïd bronchitis.

SUMMARY

Seventy-two children with wheezing related to respiratory tract infections have been investi-

gated regarding virus serology and serum IgE levels. Positive virus serology with the CF technique was achieved in 18% to RS virus in spite of absence of epidemics. Seroconversion to ECHO viruses types 3, 6, 7, 9 and 11 was seen in altogether 18%. Antibody titers indicating infections with parainfluenzavirus, adenovirus or influenza A 2 virus were found less frequently. Twenty-one of the infants had high IgE levels and these infants had allergic heredity, recidivating symptoms, former atopic manifestations and eosinophilia in blood and secretions more often than the infants with normal IgE. Positive virus serology was found as often in the infants with high IgE as in those with normal IgE. Determinations of the serum IgE levels seem to be of value in assessing the possible atopic component in children with asthmatoïd bronchitis.

ACKNOWLEDGEMENTS

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parainfluenza infection which had provoked their wheezing

Adenoviruses are held to be a rather common cause of bronchitis and pneumonia in infants. On the other hand these viruses seldom cause bronchiolitis. Hornsleth (18) found positive serology to adenovirus in 6-8% of pneumonia in children, while Chirnock & Parrott (6) and Lodz et al (28) found positive serology to this virus in only 2.4% and 1% respectively in children with bronchiolitis. In 2 of our 3 children with titer rises to adenovirus there was a simultaneous titer rise against another virus. The clinical significance of these adenovirus titers must therefore be regarded as uncertain.

ECHO viruses as a cause of wheezing has not been frequently reported. It is probably due to difficulties in the serological diagnosis. No evidence of crossreaction with Herpes simplex virus was found in this study.

During the winter 1968-69 there was an epidemic of influenza A-2 (Hongkong) in the population. In spite of this there was only one child in our study with a titer rise against this virus.

Other less common causes of wheezing in infants and children such as Mycoplasma pneumoniae and rhinovirus have not been investigated. This was because the principal aim of the study was to get an understanding of the distribution of the most common virus types in asthmatic bronchitis in relation to a possible atopy in the child.

Infections with bronchiolotropic viruses are considered to give a swelling of the mucous membranes which along with the increased secretions cause obstructive bronchial symptoms. The symptoms are accentuated in infants by the compression of their delicate and weak bronchial tree that accompanies the increased intrathoracic pressure during the forced expiration. The view that bronchial obstruction occurring in virus infected children is merely of a non specific mechanical nature is held by Simon & Jordan (37). In a study of children with bronchiolitis, they concluded that children with a demonstrable RS virus infection had a

smaller risk of developing bronchial asthma than those where such an infection could not be demonstrated.

It is noteworthy, however, that RS virus infected infants so often develop severe respiratory tract infections despite the presence of maternal neutralizing antibodies (8). RS vaccinated infants often become sicker than non vaccinated infants when infected with RS virus (25). These data have led some authors to suspect an immunologic background to the symptoms in the children having antibodies to RS virus at the onset of a RS virus infection (8, 15). However, no definite relationship has been found in this study between the appearance of a certain virus type and an atopic constitution of the child.

A high level of IgE in serum has been noted in about 60% of children with extrinsic asthma, and in about 30% of children with allergic rhino-conjunctivitis (3). Other diseases in which high IgE levels have been found are atopic eczema and certain parasitic diseases (24). In northern latitudes where parasitic diseases are rare, a high serum level of IgE probably indicates atopy. A normal or low IgE level does not exclude an atopic disease however. If the child has symptoms only during a certain period of the year the serum IgE may be increased during this time but return to the normal during the rest of the year (3). Even children with non seasonal extrinsic asthma may have normal IgE levels. Different allergens seem to stimulate reagin production to a varying degree. Dust and moulds in this respect seem to be weak allergens and this can perhaps explain why some asthmatic children have normal IgE levels (3).

In our study we found serum IgE levels exceeding 2 S.D. of the mean for age in about 30% (21 of 72 cases). These high IgE levels are probably an expression of atopy with a production of reaginic antibodies. Evidence for atopy in these children compared to the children with normal or low IgE levels was the high frequency of such factors that are held to be prognostically unfavourable (Table 2).

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such as malnutrition, malabsorption or skeletal, kidney or endocrine disorders. Thus the material was regarded as normal with respect to the calcium distribution in plasma.

METHOD

Principle of the method It is well known that pH and temperature influence the distribution of calcium between ionized and the protein bound fractions. Therefore these factors were controlled. The ultrafiltrate was prepared by an over pressure and the Ca/K ratio of the ultrafiltrate was determined. The determination of the ultrafiltrable calcium (Ca_{uf}) was made on the basis of the formula

$$Ca_{uf} = \frac{Ca_f \cdot K_f}{K_i}$$

in which Ca_{uf} = ultrafiltrable calcium in mg/100 ml plasma, Ca_f = calcium in mg/100 ml ultrafiltrate, K_i = potassium in mmol/l plasma, K_f = potassium in mmol/l ultrafiltrate.

Thus the method presupposes that the Ca/K ratio in the ultrafiltrate is equal to the ratio between ultrafiltrable calcium in plasma and plasma potassium.

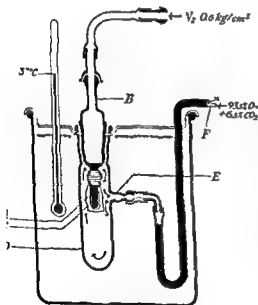


Fig. 1. The ultrafiltration set. A = collodion membrane, B = adapter with a B 29 cone for tube D and an upper 18/19 socket ball. C = plastic bag loosely tied around the collodion membrane and with some small holes in its upper part. D = test tube with side outlet = E. F = plastic tubing inlet for the CO₂-O₂ gas. The black outer plastic tubing works as an outlet for the gas.

Preparation of the samples Blood was drawn from fasting subjects and was taken at minor venous stasis (not exceeding 50 mmHg) and collected in heparin tubes. Umbilical vein blood was drawn at birth within five minutes after birth. Plasma was separated by centrifugation. A small sample was used to determine the concentration of calcium and potassium. The remaining sample was stored at +37°C.

Ultrafiltration apparatus Ultrafiltration was performed in the apparatus shown in Fig. 1. The apparatus was a modification of the one described by Rose (2). A collodion shell (Sartorius membranfilter GmbH No 13200 Membranfiltergesellschaft Göttingen West Germany) was shortened by cutting off its upper part and using the lowest 2.5 cm. This sac (A) was attached to the funnel shaped lower part of the adapter (B) which was composed of a B 29 cone and an upper 18/19 socket ball (Quickfit). The sac was put into a plastic bag (C) which was about 3 cm long and which had a somewhat larger diameter than the collodion sac and a few small holes in its upper part. The plastic bag was loosely tied around the collodion sac. An 11×3 cm test tube (D) with a B 29 socket was attached to the adapter. The test tube had a side outlet (E) which was lengthened with a rubber tubing through which a thin plastic tubing (F) was inserted and connected to a gas cylinder (93.3 O + 6.5 CO₂). The apparatus was submerged into a water bath at +37°C as shown in Fig. 1. After adding plasma as described below the socket ball of the adapter (B) was attached to a cone cup S 19 (Quickfit) connected to a nitrogen cylinder and a pressure gauge. Between runs the collodion sac was stored in saline.

Procedure 1.5–2 ml plasma was transferred to the collodion sac (A). The CO₂-O₂ mixture was supplied through the thin plastic tubing (F). After temperature equilibration to +37°C the adapter (B) was connected to the nitrogen cylinder and a pressure of 0.6 kg/cm² was applied.

An ultrafiltrate soon appeared in the plastic bag (C) in contact with the collodion sac (A). 0.5 ml required about 10–15 min and was sufficient for duplicate determinations of calcium and potassium at the same dilution (1:20) in an Eppendorf flame photometer. The plasma residue in the collodion sac (A) was used for pH determination with a Radiometer capillary electrode type G 97/92 (accuracy ±0.01 pH unit at constant temperature).

COMMENTS ON THE METHOD

Measurements of pH before and after the ultrafiltration procedure showed a very small mean change of ±0.01 units when the CO₂-O₂ mixture was applied continuously. It is thus sufficient to determine the pH of the residue in the collodion sac after ultrafiltration as described in the procedure.

PLASMA CALCIUM FRACTIONS IN NORMAL SUBJECTS FROM BIRTH TO ADULT AGES

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Total calcium in blood plasma appears in three different fractions: ionized, protein bound and complexed (as citrate, phosphate, sulphate and carbonate). The ionized calcium is kept within narrow limits by the regulatory function of the parathyroid glands and of the hormone thyrocalcitonin from the parafollicular cells in the thyroid gland. Complexed calcium is a small fraction and has not been subjected to intensive study. Ultrafiltrable calcium comprises ionized and complexed calcium.

The amount of calcium bound to the plasma proteins is said to be determined primarily by the concentrations of ionized calcium and plasma proteins. Thus, there are those who maintain that the determination of ionized calcium or ultrafiltrable calcium adds nothing to the information gained from the determination of the total plasma calcium, at least not in patients with endocrine disorders (14). Other authors, however, are of the opinion that different protein fractions have different capacities to bind calcium (8, 10). Furthermore it has been pointed out that ultrafiltrable and protein bound calcium may deviate from the normal distribution under different clinical conditions (6, 25). Bronner (2) concluded that our knowledge in this field is incomplete. Consequently he preferred direct measurement of ultrafiltrable calcium to calculation on the basis

of the nomogram of McLean & Hastings (15) which is based on the total calcium and the total plasma protein concentrations.

Numerous reports on the different calcium fractions in plasma of adults have been published. So far, however, we have found only one report (24) on the normal distribution of the calcium fractions in plasma from children of different ages. Therefore the aim of the present investigation was to study the changes of the calcium fractions from birth up to adult ages. A simple and rapid method to determine ultrafiltrable calcium in plasma was developed for this purpose and will be described.

MATERIAL

The material comprised 202 children aged 0-16 years (for age distribution see Figs 3 and 4) and 93 healthy male blood donors aged 18-62 years. The material was divided into age groups as shown in Figs 3 and 4. Sixty-six children were studied during their first week of life in a maternity ward. Delivery of all the infants was spontaneous, vaginal and without complication. None of them suffered asphyxia and the neonatal periods were uneventful. They were all breast fed beginning after 24 hours of life. Of the 14 children from 4 months up to one year, seven were patients at an orphanage. The remaining 129 subjects had been admitted to the Children's Hospital of Göteborg for minor disorders: usually phimosis, retention tests or umbilical hernia. No child had a disorder that could be expected to disturb the calcium, phosphorus or protein metabolism.

value for which the limits are sought and the variation around the regression. As the calculations are based on the assumption that there is a linear relationship within the intervals chosen and as the error inherent in the estimation of the independent variate is neglected the fiducial limits obtained are only approximate. The present results indicate that pH has its greatest effect on the distribution of plasma calcium within the pH range compatible with life. In that range our curve was much steeper than the ones reported earlier (9, 13).

The physiological range for total plasma calcium concentration is known to be narrow hence the range of ultrafiltrable calcium should be even more narrow. However if the pH is not taken into full account the standard deviation for ultrafiltrable calcium concentration in plasma will be more than twice that of total plasma calcium. When the pH is taken fully into account the standard deviation for ultrafiltrable calcium will be reduced to the same magnitude (± 0.23 mg/100 ml) as that for total plasma calcium. Thus it is of utmost importance to know plasma pH and keep it constant during the ultrafiltration. For the sake of comparison it might be better to recalculate all values to one and the same pH say 7.40 by consulting the curve in Fig. 2.

The error (coefficient of variation) for Ca_p and K_p was 0.8 ($n=93$). The error (coefficient of variation) for the Ca_f determination was calculated on the basis of duplicate determinations and was found to be 1.5 when two different membranes were used ($n=25$) and 1.3 when one membrane was used ($n=173$).

RESULTS

Figs. 3 and 4 show the distribution of plasma calcium in the different age groups. No significant differences were found between males and females in childhood. Therefore mean values and standard deviations for total calcium, ultrafiltrable calcium and protein bound

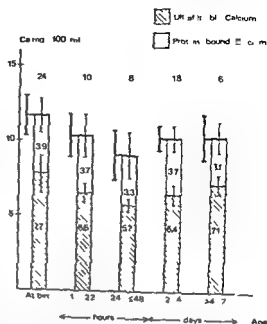


Fig. 3 Distribution of plasma calcium during the first week of life. Sixty-six normal cases. Mean values and standard deviations for total calcium (thick bars) ultrafiltrable calcium and protein bound calcium are given. All values corrected to pH 7.4.

calcium are given without regard to sex.

Total plasma calcium showed a significantly higher mean value in umbilical cord blood (11.6 mg/100 ml) than in all other age groups ($p < 0.01$). At 24-48 hours of life there was a decrease to a mean of 9.0 mg/100 ml. Later an increase in the total calcium was seen up to three months of age ($p < 0.01$). The mean values found between three months and up to one year of age were significantly higher than those seen after six years of age ($p < 0.01$). All age groups up to 16 years except for the group 24-48 hours showed significantly higher total plasma calcium levels than adults ($p < 0.01$).

Ultrafiltrable calcium (recalculated to pH 7.4) in umbilical cord blood plasma was significantly higher (mean 7.7 mg/100 ml) than in all other age groups ($p < 0.01$). After birth there was a decrease of the Ca_f fraction which reached its minimum value at 24-48 hours after birth (5.7 mg/100 ml). The mean value at 1-21 h of life was significantly higher ($p < 0.01$) than the mean value at 24-48 h. An

In previous methods, ultrafiltrable calcium has been determined on the basis of a direct analysis of calcium in the ultrafiltrate.

Transformation of the calcium concentration of the ultrafiltrate into the plasma concentration requires a correction for plasma protein volume and possibly also for the Donnan effect. The latter correction may not be reliable as the pressure applied during ultrafiltration does not lead to equilibrium which is one prerequisite for the Donnan membrane theory. With the present method no consideration has to be taken to any of these corrections as the calculation is based on a ratio between the two components in the ultrafiltrate (Ca/K). Moreover, no consideration has to be taken to possible dilution caused by water remaining in the pores of the collodion sac, nor to inaccurate dilution of the sample for the flame photometric determination.

The prerequisite for the present method is that no part of the plasma potassium is bound to plasma protein. Our results are in agreement with the view that all plasma potassium is ultrafiltrable. In carefully handled samples from our normal material, the potassium concentration in the ultrafiltrate corresponded well

with the potassium concentration in plasma $K_f = 0.96 K_p + 0.11$. Moreover, if a part of the plasma potassium were protein bound, our calculation procedure should give erroneously high values for Ca_f . Our values for adults, however, agree with those given in recent reports (1, 13, 18, 21) and are below those of older studies (9, 19, 22, 27).

The relationship between pH and ultrafiltrable calcium for the 93 healthy adult blood donors is shown in Fig. 2. Each dot represents the mean of two determinations on the same ultrafiltrate. In order to study the relation between pH and Ca_f outside the physiological pH range 0.05–0.20 M HCl or M NaOH was added to 15 ml plasma (80 samples).

Fig. 2 shows the fiducial intervals around the regression line. This line embraces the area within which 95% of individual values for the pH range 7.1–7.6 are expected to fall (4). The calculation was made according to the formula

$$y = \bar{y} \pm t \sqrt{s^2 \left(1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum (x - \bar{x})^2} \right)}$$

in which t is the 0.05 probability deviate with $n-1$ degrees of freedom, x is any particular

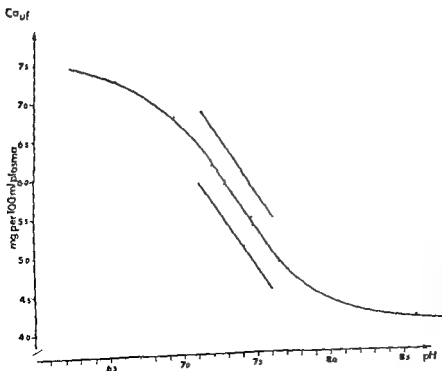


Fig. 2. Ultrafiltrable calcium in mg/100 ml plasma at different pH and +37°C. Ninety-three normal cases. The pH values outside the physiological interval were obtained by adding HCl and NaOH.

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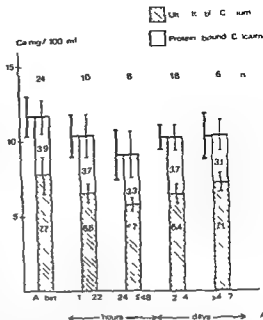


Fig. 3. Distribution of plasma calcium during the first week of life. Sixty-eight normal cases. Mean values and standard deviations for total calcium (thick bars), ultrafiltrable calcium and protein bound calcium are given. All values corrected to pH 7.4.

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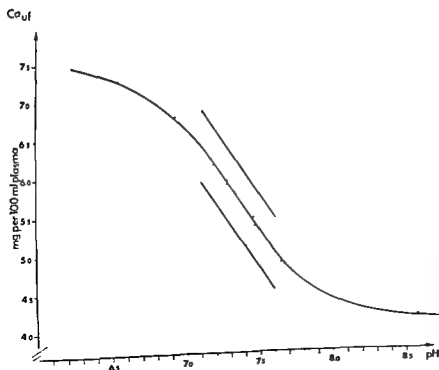


Fig 2 Ultrafiltrable calcium in mg 100 ml plasma at different pH and +37°C. Ninety-three normal cases. The pH values outside the physiological interval were obtained by adding HCl and NaOH.

ACKNOWLEDGEMENTS

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Our mean value for umbilical vein blood is within the ranges given by these authors. Our mean value for Ca^{2+} in umbilical vein blood plasma is equal to that given earlier in the literature (5). Teh (24) found ultrafiltrable calcium to decrease with age from 65% of total calcium to 55% in adult ages during the first four weeks of life. The significant decrease in ultrafiltrable calcium seen in our material from the first week of life seems to be responsible for the entire decrease in total plasma calcium. However during the first week of life there is a decrease in protein bound calcium possibly because of the lowered amount of circulating plasma albumin (16, 17).

Thus it can be concluded that the higher total plasma calcium levels seen during childhood are caused by an increased ultrafiltrable calcium fraction. It seems reasonable to assume that this in turn indicates a positive calcium balance and skeletal mineralization.

SUMMARY

A simple and rapid method to determine ultrafiltrable calcium in plasma is described in detail. Total calcium, ultrafiltrable calcium (Ca^{2+}) and protein bound calcium in plasma are reported for 202 normal children (range 0-16 years of age) divided into 12 age groups as well as for 93 healthy male blood donors. There was a decrease in plasma calcium and ultrafiltrable calcium after 1-21 hours of life. The minimum value was reached at 24-48 hours of life. An increase was then seen during the first week of life. Later in childhood total calcium and Ca^{2+} decreased continuously and significantly with age and with the most marked rate during the first two-three years.

The ultrafiltrable calcium fraction was found to be responsible for the changes in total plasma calcium. In 24-48 hours of life and 5-7 days of life protein bound calcium was significantly lower as well. There were no significant differences between males and females in childhood.

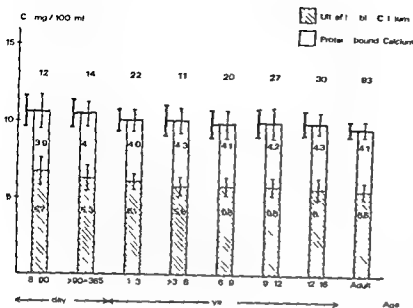


FIG. 4. Distribution of plasma calcium after the first week of life up to adults. 229 normal cases. Mean values and standard deviations for total calcium (thick bars), ultrafiltrable calcium and protein bound calcium are given. All values corrected to pH 7.4.

increase was then seen with significant difference between the 2-4 day group and the 24-48 h group ($p < 0.01$) as well as between the 4-7 day group and the 2-4 day group ($p < 0.01$). Up to three years of age, the ultrafiltrable calcium fractions were significantly higher than in the 3-6 year group ($p < 0.05$) and later in life ($p < 0.01$). Up to twelve years of age, the level was still significantly higher than in the adults ($p < 0.01$).

Protein bound calcium showed its significantly lowest value at 24-48 hours of life and at the end of the first week of life ($p < 0.01$). Later there were some significant differences between the groups, however, without any certain tendency.

DISCUSSION

The technique used for blood sampling varied with the age group. No venous stasis was applied before three years of age. At birth blood was drawn from vena umbilicalis. Up to one year of age blood was drawn from vena jugularis interna. From one year to three years of age, the blood was drawn from vena femoralis, and after three years from the cubital vein. Venous stasis above a certain pressure is known to induce an *in vivo* ultrafiltration which should mean a rise in total plasma calcium

(12). The venous stasis used by us was below the critical level. During the first month of life, the meals are so frequent that a true fasting state does not exist. This can partly account for the increased plasma calcium levels in these ages. The number of meals then decreases to five per day and is then further reduced to four a day at four months of age. The significance of this is difficult to determine.

Total plasma calcium has been reported to be higher in children than in adults already shown in 1921 (11). Later reports on this subject however are rare and only one (24) reports a variation in total plasma calcium and Ca_{10} with age. According to Teh (24) only blood collected at birth contains significantly higher amounts of total calcium than the blood in adults. During the first week of life, the level was significantly lower than in adult life. All of our values are higher than those of Teh (24) and thus more in conformity with the older concept. The decrease in total plasma calcium during the first week of life has been reported earlier (3, 20, 23). The minimum level found by us is in agreement with recent reports (7, 28). Several investigators (for references, see 5, 26) have found that total plasma calcium in umbilical cord is higher than in maternal plasma.

METABOLIC ALKALOSIS IN INFANTS. ROLE OF WATER DEPLETION AND CHANGES IN COMPOSITION OF STOOL

Review of a Physiological Problem

POUL KILDEBERG and KNUD ENGBL

*From the Departments of Paediatrics Clinical Chemistry and Physiology
Odense University Medical School Odense Denmark*

the pathophysiologic interpretation of any metabolic alkalosis two obligatory requirements must be met firstly the induction of the disturbance must be accounted for by identifying a quantitatively meaningful net gain of titratable base secondly the maintenance of the disturbance must be accounted for by identifying the factor(s) instrumental in the associated rise in the renal bicarbonate threshold the rise in the rate of generation of bicarbonate by the renal tubular epithelium

In recent years a number of determinants of the rate of tubular bicarbonate generation have been studied under both experimental and clinical conditions These include the arterial pH , mineralocorticoid hormones and stores of potassium and chloride and it appears that a reduced rate of glomerular filtration of chloride is an important factor in the maintenance of gastric alkalosis (4) On the other hand limited attention has been paid to the question of the source of the priming net load of base the present paper purports to examine this problem in the light of new developments in the area of gastrointestinal acid base physiology specifically the role of gastric hydrochloric acid depletion will be reconsidered in the light of obligatory effects of concomitant changes in the balance of water

It is generally held that in prolonged obstructive vomiting depletion of gastric hydrochloric acid is responsible for the induction of the alkalotic disturbance This view is simple and fits the Bronsted formulation of acid base chemistry well It will be observed however that sustained losses of hydrochloric acid from the stomach (which do occur in pyloric stenosis) do not necessarily imply net losses of acid from the body a point which has attracted few acid base physiologists Similarly although the phenomena of dilution acidosis and concentration alkalosis have received some attention (1-13) the implications of the underlying principle for our understanding of several common clinical acid base disturbances especially post vomiting alkalosis appear to have been overlooked

Open and closed titration systems the titratable value of water

Acid base titration involves evaluation of concentrations of acid or base in terms of quantities of hydroxyl ion or hydrogen ion added per unit of sample volume in order to produce a specified change in pH Titrating bicarbonate containing buffer solutions like the body fluids requires additional specifications of any change in P_{CO_2} and total CO_2 concentration associated with the titration Titration at a constant total CO_2 concentration (closed system titration) is

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heavily acidotic blood but very appreciable for samples with BE levels in the alkalotic range. Results of actual mixing experiments for $\lambda = 1$ are presented in Fig. 1.

In chemistry, acid base titrations are carried out to an arbitrary end point at pH 7.00 (temperature 25°C, P_{CO_2} zero mmHg) the advantage being that solutes can be titrated with negligible interference by the aqueous medium. Thus the conventional acid base diagnostic system using average normal (adult) blood acid base values as the arbitrary basis for statements concerning titratable values may seem unnecessarily complicated. It does however constitute a frame of reference within which the effects on the pH of the body fluids of exchanges of acid (base) and water can and should, be explored separately.

Implications for blood acid base status in obstructive vomiting

That the order of magnitude of the dilutional effect of water on the blood BE demonstrated in Fig. 1 may apply to *in vivo* alkalosis can be realized from the following hypothetical examples.

Consider a four kilogram infant with 350 ml/kg of extracellular fluid (3). Following prolonged obstructive vomiting the water deficit may reach 15% of the body weight (11) i.e. 600 ml and the blood BE may rise to +8 mEq/l (5). Assuming the dehydration to be iso-osmolar (5, 12) the contraction will be confined largely to the extracellular space. Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for *in vivo* bicarbonate redistribution) 800 ml of extracellular fluid will be left containing $0.8 \times 8 = 6.4$ milliequivalents of titratable base. Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing $0.6 \times 25.5 = 15.3$ milliequivalents of titratable acid the 1400 ml of extracellular fluid will now contain only 0.7 milliequivalents of titratable base which means that the blood BE will have fallen to +0.5 mEq/l.

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be +6.0 mEq/l. Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1.5 mEq/l).

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can

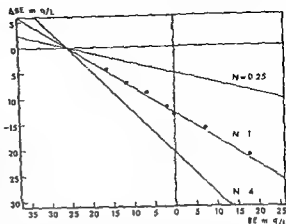


Fig. 1 Changes in blood base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE. Solid lines represent equation (v) for various values of N . Dots represent results of mixing experiments for $N=1$ using 0.9% sodium chloride as the diluent.

be conceived as a mere consequence of extra cellular fluid contraction. Obviously such considerations do not permit of the conclusion that a negative water balance is the sole primary cause of elevation of the BE of blood in obstructive vomiting. They do in our opinion suffice to establish water loss as one major determinant as well as to point to the need for a closer view on the relation between gastric HCl depletion and the net input of base from the gastrointestinal tract. The latter can be studied conveniently in terms of a titration end point at pH 7.40 and P_{CO_2} zero mmHg.

Vomiting and gastrointestinal acid base exchange

The parietal cells of the gastric glands generate hydrogen ions. These are secreted into the lumen at the expense of the equivalent quantity of hydroxyl ion being added (as bicarbonate) to the venous return. Similarly the pancreatic acini secrete bicarbonate at a rate equivalent to the rate of acidification of the pancreatic venous blood. The jejunal glands like the gastric epithelium produce an acid juice charging the blood with base at the equivalent rate (8, 11) whereas finally the ileal and colonic secretions are alkaline (9). In so far as direct ionic

essentially similar to titration of bicarbonate-free buffer systems but, for methodological reasons, is rarely practicable. Procedures allowing for CO_2 exchange with the environment (open system titration) actually involve titration of the sample with CO_2 as well as with the titrant. In such cases, however, the titratable acidity may still be expressed in terms of quantities of (non carbonic) titrant added but specification of the end point Pco is clearly called for.

Consider an λ millimolar solution of NaOH titrated with HCl to pH 7.40. Representing the initial pH by x the result of simple titration at Pco zero mmHg may be stated as follows (TA denoting titratable acid)

$$[\text{TA}]_{\text{pH } 7.40 \text{ Pco } 0} - [\text{TA}]_{\text{pH } x \text{ Pco } 0} = -X \text{ mEq/l} \quad (i)$$

Titration of the same NaOH solution to pH 7.40 and Pco 40 mmHg however involves CO_2 absorption and the generation of bicarbonate $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$. Taking the pK_A of carbonic acid and the CO_2 absorption coefficient to be 6.10 and 0.032 respectively for pure water at body temperature (10) the Henderson-Hasselbalch equation shows that 25.5 mmol/l of bicarbonate have been formed when the end point is reached

$$7.40 = 6.10 + \log \frac{25.5}{0.032 \times 40}$$

Consequently only $\lambda - 25.5$ mmol/l of HCl will be required to reach pH 7.40 the remaining hydroxyl ions being titrated with CO_2

$$[\text{TA}]_{\text{pH } 7.40 \text{ Pco } 40} - [\text{TA}]_{\text{pH } x \text{ Pco } 0} = 25.5 - Y \text{ mEq/l} \quad (ii)$$

In a phosphate bicarbonate buffer in equilibrium at pH 7.40 and Pco 40 mmHg gain of Z mmol/l of NaOH at an unchanged Pco will lead to CO_2 absorption. Upon subsequent titration with HCl the same quantity of CO_2 will be released hence

$$[\text{TA}]_{\text{pH } 7.40 \text{ Pco } 40} - [\text{TA}]_{\text{pH } z \text{ Pco } 40} = -Z \text{ mEq/l} \quad (iii)$$

where z represents the sample pH following gain of Z mmol/l of NaOH . For closed system titrations an expression analogous to (i) and (iii) obtains

In general for any acid base system a family of parallel TA scales exist for each end point pH. These scales conform to the general equation $\text{TA} = n - Y$ where Y denotes milliequivalents of base accumulated including any bicarbonate. The significance of n becomes clear when X assumes the value of zero in which case $\text{TA} = n$. Thus n is the titratable value of the solvent i.e. water. For example titrating water from pH 7.40 (assuming a trace of NaOH to be present initially) at Pco zero mmHg to pH 7.40 at

Pco 40 mmHg involves the addition of 25.5 mmol of NaOH to each liter of water

$$[\text{TA}]_{\text{pH } 7.40 \text{ Pco } 40} - [\text{TA}]_{\text{pH } 7.40 \text{ Pco } 0} = 25.5 \text{ mEq/l} \quad (iv)$$

We may now rewrite equation (ii) in these terms

$$[\text{TA}]_{\text{pH } 7.40 \text{ Pco } 40} - [\text{TA}]_{\text{pH } z \text{ Pco } 40} = 25.5 - (Z + 25.5) \text{ mEq/l} \quad (v)$$

In this formulation equation (iii) represents the "base excess" (BE) scale of blood cf (10)

It follows that with respect to the particular end point considered pH 7.40 at Pco 40 mmHg water must be regarded an acid, albeit in molar terms an exceedingly weak one. Whereas one mole of HCl represents one mole of titratable hydrogen ion (i.e., one equivalent), one mole of water (18 ml) represents only $0.0255 \times 10^{-3} \times 18 = 0.00046$ moles of titratable hydrogen ion. Furthermore, whereas the volume effect of strong acid or base per se accumulating within the limits of tolerance of the body is negligible, the volume effect of water is substantial. Hence the amount of strong acid or base gained in conditions of a zero water balance is directly related to the resulting change in the concentration of TA in the body fluids whereas the effect of a given water load depends on the pre-existing level of the latter. Provided the TA of the body fluids is less than 25.5 mEq/l, water accumulation will cause the TA to rise. If however, the body fluid TA is above 25.5 mEq/l (extreme metabolic acidosis) a water load will cause the TA to fall. A quantitative formulation of this effect can be derived from a few numerical examples.

Mixing equal parts of normal blood ($\text{TA} = -\text{BE} = \text{zero mEq/l}$) and isotonic sodium chloride solution ($\text{TA} = 25.5 \text{ mEq/l}$) results in a change in the blood BE from zero to -12.75 mEq/l . Mixing one part of blood with a BE of $+25.5 \text{ mEq/l}$ and two parts of isotonic saline produces a fall in blood BE from $+25.5$ to -8.5 mEq/l etc. Hence the calculated effect of the initial blood BE on the change in blood BE resulting from mixing blood and water (e.g. as 0.9% NaCl) can be formulated as follows

$$\Delta \text{BE} = -\frac{N}{N+1} (25.5 + \text{BE}_{\text{initial}}) \quad (vi)$$

where N is the number of parts of water added to one part of blood. Clearly the effect is small for

heavily acidotic blood but very appreciable for samples with BE levels in the alkalotic range. Results of actual mixing experiments for $N=1$ are presented in Fig. 1.

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Consider a four kilogram infant with 350 ml/kg of extracellular fluid (3). Following prolonged obstructive vomiting the water deficit may reach 15% of the body weight (17), i.e. 600 ml and the blood BE may rise to +0 mEq/l (5). Assuming the dehydration to be iso-osmolar (5, 1) the contraction will be confined largely to the extracellular space. Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for *in vivo* bicarbonate redistribution) 800 ml of extracellular fluid will be left containing 0.8 \times 16 milliequivalents of titratable base. Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing $0.6 \times 15.5 = 9.3$ milliequivalents of titratable acid the 1400 ml of extracellular fluid will now contain only 17 milliequivalents of titratable base which means that the blood BE will have fallen to +0.5 mEq/l.

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be +6.0 mEq/l. Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1.5 mEq/l).

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can

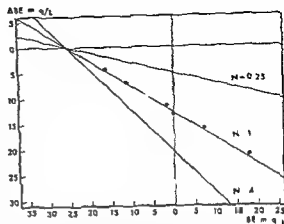


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Consider an $\frac{1}{10}$ millimolar solution of NaOH titrated with HCl to pH 7.40. Representing the initial pH by λ , the result of simple titration at P_{CO_2} zero mmHg may be stated as follows (TA denoting titratable acid)

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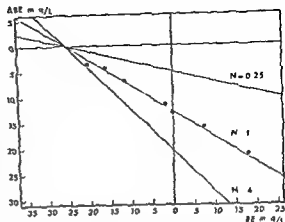


Fig 1 Changes in blood "base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE. Solid lines represent equation (y) for various values of N . Dots represent results of mixing experiments for $N=1$ using 0.9% sodium chloride as the diluent.

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The parietal cells of the gastric glands generate hydrogen ions. These are secreted into the lumen at the expense of the equivalent quantity of hydroxyl ion being added (as bicarbonate) to the venous return. Similarly the pancreatic acini secrete bicarbonate at a rate equivalent to the rate of acidification of the pancreatic venous blood. The jejunal glands like the gastric epithelium produce an acid juice charging the blood with base at the equivalent rate (8, 11) whereas finally the ileal and colonic secretions are alkaline (9). In so far as direct ionic

essentially similar to titration of bicarbonate-free buffer systems but, for methodological reasons, is rarely practicable. Procedures allowing for CO_2 exchange with the environment (open system titration) actually involve titration of the sample with CO_2 as well as with the titrant. In such cases, however, the titratable acidity may still be expressed in terms of quantities of (non carbonic) titrant added, but specification of the end point P_{CO_2} is clearly called for.

Consider an X millimolar solution of NaOH titrated with HCl to $\text{pH } 7.40$. Representing the initial pH by τ the result of simple titration at P_{CO_2} zero mmHg may be stated as follows (TA denoting titratable acid)

$$[\text{TA}]_{\text{pH } 7.40, P_{\text{CO}_2} 0} = -X \text{ mEq/l} \quad (i)$$

Titration of the same NaOH solution to $\text{pH } 7.40$ and P_{CO_2} 40 mmHg however involves CO_2 absorption and the generation of bicarbonate $\text{CO} + \text{OH}^- \rightarrow \text{HCO}_3^-$. Taking the pK_A of carbonic acid and the CO_2 absorption coefficient to be 6.10 and 0.032 respectively for pure water at body temperature (10) the Henderson-Hasselbalch equation shows that 25.5 mmol/l of bicarbonate have been formed when the end point is reached

$$7.40 = 6.10 + \log \frac{25.5}{0.032 \times 40}$$

Consequently only $1 - 25.5$ mmol/l of HCl will be required to reach $\text{pH } 7.40$ the remaining hydroxyl ions being titrated with CO_2

$$[\text{TA}]_{\text{pH } 7.40, P_{\text{CO}_2} 40} = 25.5 - X \text{ mEq/l} \quad (ii)$$

In a phosphate bicarbonate buffer in equilibrium at $\text{pH } 7.40$ and P_{CO_2} 40 mmHg gain of Z mmol/l of NaOH at an unchanged P_{CO_2} will lead to CO_2 absorption. Upon subsequent titration with HCl the same quantity of CO_2 will be released hence

$$[\text{TA}]_{\text{pH } 7.40, P_{\text{CO}_2} 40} = -Z \text{ mEq/l} \quad (iii)$$

where n represents the sample pH following gain of Z mmol/l of NaOH . For closed system titrations an expression analogous to (i) and (iii) obtains.

In general for any acid base system a family of parallel TA scales exist for each end point pH . These scales conform to the general equation $\text{TA} = n - X$ where X denotes milliequivalents of base accumulated including any bicarbonate. The significance of n becomes clear when X assumes the value of zero in which case $\text{TA} = n$. Thus n is the titratable value of the solvent i.e. water. For example titrating water from $\text{pH } 7.40$ (assuming a trace of NaOH to be present initially) at P_{CO_2} zero mmHg to $\text{pH } 7.40$ at

P_{CO_2} 40 mmHg involves the addition of 25.5 mmol of NaOH to each liter of water

$$[\text{TA}]_{\text{pH } 7.40, P_{\text{CO}_2} 40} - 25.5 \text{ mEq/l} \quad (iv)$$

We may now rewrite equation (iii) in these terms

$$[\text{TA}]_{\text{pH } 7.40, P_{\text{CO}_2} 40} = 25.5 - (Z + 25.5) \text{ mEq/l} \quad (v)$$

In this formulation equation (iii) represents the base excess (BE) scale of blood of (10)

It follows that with respect to the particular end point considered $\text{pH } 7.40$ at P_{CO_2} 40 mmHg water must be regarded an acid albeit, in molar terms, an exceedingly weak one. Whereas one mole of HCl represents one mole of titratable hydrogen ion (i.e., one equivalent), one mole of water (18 ml) represents only $0.0255 \times 10^{-3} \times 18 = 0.00046$ moles of titratable hydrogen ion. Furthermore, whereas the volume effect of strong acid or base per se, accumulating within the limits of tolerance of the body, is negligible, the volume effect of water is substantial. Hence the amount of strong acid or base gained in conditions of a zero water balance is directly related to the resulting change in the concentration of TA in the body fluids, whereas the effect of a given water load depends on the pre-existing level of the latter. Provided the TA of the body fluids is less than 25.5 mEq/l water accumulation will cause the TA to rise. If however the body fluid TA is above 25.5 mEq/l (extreme metabolic acidosis) a water load will cause the TA to fall. A quantitative formulation of this effect can be derived from a few numerical examples.

Mixing equal parts of normal blood ($\text{TA} = -\text{BE} = \text{zero mEq/l}$) and isotonic sodium chloride solution ($\text{TA} = 25.5 \text{ mEq/l}$) results in a change in the blood BE from zero to -12.75 mEq/l . Mixing one part of blood with a BE of $+25.5 \text{ mEq/l}$ and two parts of isotonic saline produces a fall in blood BE from $+25.5$ to -8.5 mEq/l etc. Hence the calculated effect of the initial blood BE on the change in blood BE resulting from mixing blood and water (e.g. as 0.9 NaCl) can be formulated as follows

$$\Delta \text{BE} = -\frac{N}{N+1} (25.5 + \text{BE}_{\text{initial}}) \quad (v)$$

where N is the number of parts of water added to one part of blood. Clearly the effect is small for

heavily acidotic blood but very appreciable for samples with BE levels in the alkalotic range. Results of actual mixing experiments for $N=1$ are presented in Fig. 1.

In chemistry, acid base titrations are carried out to an arbitrary end point at pH 7.00 (temperature 25°C, P_{CO_2} zero mmHg) the advantage being that solutes can be titrated with negligible interference by the aqueous medium. Thus the conventional acid base diagnostic system using average normal (adult) blood acid base values as the arbitrary basis for statements concerning titratable values may seem unnecessarily complicated. It does however constitute a frame of reference within which the effects on the pH of the body fluids of exchangers of acid (base) and water can and should be explored separately.

Implications for blood acid base status in obstructive vomiting

That the order of magnitude of the dilutional effect of water on the blood BE demonstrated in Fig. 1 may apply to *in vivo* alkalosis can be realized from the following hypothetical examples.

Consider a four kilogram infant with 350 ml/kg of extracellular fluid (3). Following prolonged obstructive vomiting the water deficit may reach 15% of the body weight (1) i.e. 600 ml and the blood BE may rise to +20 mEq/l (5). Assuming the dehydration to be iso-osmolar (5, 17) the contraction will be confined largely to the extracellular space. Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for *in vivo* bicarbonate redistribution) 800 ml of extracellular fluid will be left containing $0.8 \times 20 = 16$ milliequivalents of titratable base. Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing $0.6 \times 75.5 = 45.3$ milliequivalents of titratable acid the 1400 ml of extracellular fluid will now contain only 0.7 milliequivalents of titratable base which means that the blood BE will have fallen to +0.5 mEq/l.

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be +6.0 mEq/l. Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1.5 mEq/l).

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can

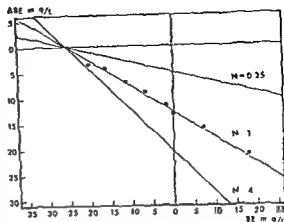


Fig. 1 Changes in blood base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE. Solid lines represent equation (4) for various values of N . Dots represent results of mixing experiments for $N=1$ using 0.9% sodium chloride as the diluent.

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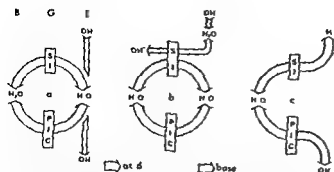


Fig 2 Suggested types of gastrointestinal acid base response *a* oral ingestion of base net zero absorption *b* oral ingestion of base net positive absorption *c* obstructive vomiting net zero absorption *S J P I C* Gastric jejunal pancreatic ileal and colonic glands respectively *B* Portal blood *G* Gastrointestinal membrane *L* Lumen

transport, from lumen to blood, plays only a minor role in gastrointestinal absorption of acid or base this serial glandular arrangement constitutes an almost perfect homeostatic system if the total secretion of hydrogen ion (by stomach and jejunum) and the total secretion of base (by pancreas and ileum colon) are maintained at equal rates there can be no net acidification of the portal blood ingested non-metabolizable acid or base being "shunted" through the gastrointestinal tract to appear in the stool (7) On the other hand, net absorption of any given quantity of base during any given period of time requires the total amount of acid secreted during that period to exceed the amount of base concurrently secreted at other levels by the identical quantity, etc (Fig 2)

Thus according to present views on the mechanics of gastric hydrochloric acid production the immediate influence on the blood acid-base status is manifest at the moment of H^+ secretion and independent of the luminal fate of the generated hydrogen ions If in the event of vomiting, excess (not neutralized) pancreatic bicarbonate is left in the lumen the development of alkalosis is contingent upon reabsorption of this amount of base To the extent that the excess base is not reabsorbed (but lost with the stool) the blood acid base status will remain unaffected But reabsorption can occur only as a result of equivalent H^+ secretion in casu

presumably by the jejunum Alternatively suppression of the rate of pancreatic bicarbonate secretion may take place as a direct consequence of gastric losses of hydrochloric acid Either way we are forced to accept the conclusion that in terms of gastrointestinal acid base physiology a positive net base balance as an immediate effect of vomiting can be conceived only as a result of net hypersecretion of acid Moreover, any homeostatic measure minimizing the rate of accumulation of base in the extracellular fluid must aim at maintaining identity between rates of secretion of acid and base at various levels of the gastrointestinal tract

That such an acid base homeostatic system is indeed operative in the gastrointestinal tract was suggested by previous studies of the balance of net acid (defined as non-carbonic, non-metabolizable acid on titration to pH 7.40 and P_{CO_2} , zero mmHg) in healthy infants (7) In these studies, an extremely close correlation was established between rates of dietary intake of net base (NB) represented by organic anion and rates of concurrent fecal net base excretion ($NB_{stool} = 0.737 \times NB_{diet} + 1.711$, $p < 0.0005$) Further studies involving changes in the composition of the diet complemented these findings (2, 6) Once more, the effect of a change in the rate of oral ingestion of base was obviated by an almost equivalent change in the rate of stool losses of base Similar data on adults are not available Conceivably the gastrointestinal tract may respond in a similar fashion to losses of hydrochloric acid from the stomach the portal venous return escaping alkalization (Fig. 2) Indeed, the gastrointestinal tract seems eminently suited to compensate for variations in the amount of acid or base offered for transport It is not suited to compensate for losses of water

SUMMARY

Simple calculations of the effect of volume depletion on the concentration of titratable base of blood show this effect to be largely adequate

to account for commonly observed degrees of metabolic alkalosis in patients with mild or extreme dehydration due to obstructive vomiting. This suggests that hydrochloric acid depletion per se may not be a major determinant of the blood acid base status in such conditions. Clearly part of the base equivalents missing in the extracellular fluid may be sought in the cell water but currently available evidence concerning gastrointestinal transport of acid and base invites a conception of gastrointestinal acid base homeostasis—and the hypothesis that in obstructive vomiting changes in the composition of stool may appreciably modify the effect of gastric hydrochloric acid depletion. In studies of intracellular buffering and net base balance in gastric alkalosis it must be realized that the primary extracellular disturbance may be largely contractional and that stool losses (or luminal pooling) of base may be an important variable.

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CONCENTRATION OF PURINE NUCLEOTIDES IN ERYTHROCYTES OF PATIENTS WITH THE LESCH-NYHAN SYNDROME BEFORE AND DURING ORAL ADMINISTRATION OF ADENINE

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Previously we reported the influence of oral adenine administration on the clinical features and purine metabolism in the Lesch-Nyhan syndrome (20). This syndrome is an X-linked recessive disorder with choreoathetosis, hyperuricaemia, mental retardation, aggressive behaviour, compulsive automutilation and occasionally megaloblastic anaemia (9, 11, 12, 14, 15). The syndrome is biochemically characterized by a deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRTase) EC 2.4.2.8 (17), and a greatly increased purine synthesis *de novo* (11, 13).

In view of the key position of the purine nucleotides in purine metabolism (Fig. 1) it seemed worthwhile to determine the content of these nucleotides in erythrocytes of two patients with the Lesch-Nyhan syndrome before and during oral administration of adenine.

MATERIAL AND METHODS

Purine nucleotides were analysed according to a modified method of Hurlbert et al. (8). The erythrocytes of 20 ml blood were haemolysed with one volume distilled water. To the obtained haemolysate 20 ml of an ice-cooled trichloroacetic acid (TCA) solution

(10%) were immediately added. This mixture was stirred vigorously and then centrifuged for 10 min at 3000 rpm. The obtained supernatant was kept in an ice-water bath. The precipitate was extracted twice with 20 ml of an ice-cooled TCA solution (5%) and centrifuged as given before. The combined supernatants were extracted 6 times with equal volumes of diethylether to remove the TCA. The resulting aqueous solution with a pH of about 6 was applied to a Dowex 1 × 2 (200-400 mesh) column (25 × 0.7 cm), equilibrated with 0.8 M ammoniumformate in 6 N formic acid. Elution was performed with two consecutive linear gradients: I distilled water to 4 N formic acid (total volume 800 ml) and II 4 N formic acid to 0.8 M ammoniumformate in 4 N formic acid (total volume 800 ml). Fractions of 5 ml were collected.

The absorbance of the eluate was continuously registered at 254 nm during the elution. The obtained peaks were identified by measurement of the absorbance at 260, 265, 270, 275 and 280 nm by paper chromatography of an aliquot and by comparison with the authentic compounds and in the case of di- and triphosphates by the determination of the labile phosphate content of these solutions according to the method of Fiske & Subbarow (4). The amount of the identified compounds was calculated from the absorbance of the concerning solutions at 265 nm against a blank obtained by mixing equal volumes of the fractions preceding and following the peak. This had been justified since a linear gradient had been applied.

Recovery experiments had been performed by addition of guanosine 5 monophosphate (GMP) and labelled adenosine triphosphate to the erythrocytes before haemolysing. A mean recovery of 96% was found. Definite calculations had been made with regard to this percentage.

In every blood sample the haemoglobin content, the haematocrit value and the erythrocyte count were determined according to our current laboratory methods.

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Table 1 Concentration of purine nucleotides in the patients Re W and Ru W before and during oral administration of adenine

	Haemato- crit	MCHC	Nucleotides in μ moles per litre of free cell water ^a			
			AMP	ADP	ATP	GTP
Normal individuals						
Mean value \pm S.D.			37.9 \pm 2.03	224 \pm 30	1 830 \pm 110	103 \pm 10
Re W						
before adenine administration	38.0	33.0	32.2	118	1 500	114
during adenine administration	37.5	32.5	41.5	229	1 980	127
Ru W						
before adenine administration	33.5	33.0	35.7	111	1 580	120
during adenine administration	39.0	36.0	40.0	228	1 960	124

^a The concentration of nucleotides per litre of free cell water is calculated according to Heim (7) by the formula

$$\text{conc./l of free cell water} = \frac{100}{100 - \text{MCHC}} \times \text{conc./l of packed cells}^b$$

^b The concentration per litre of packed cells can be calculated from the concentration per litre of whole blood and the haematocrit value

RESULTS

Table 1 shows the result obtained with erythrocytes of 12 children of varying ages without metabolic haematological neurological or mental disease and of two brothers Re W (9 years) and Ru W (2 years) suffering from the Lesch Nyhan syndrome.

In both patients purine nucleotides had been determined before and after 32 weeks of oral administration of adenine. During this period Re W obtained 200 mg (13 mg/kg) of adenine daily. Ru W obtained during the first 7 weeks 160 mg daily (14 mg/kg) and the following 25 weeks 240 mg daily (22 mg/kg).

The following nucleotides had been identified in erythrocytes: adenosine 5 monophosphate (AMP), adenosine-diphosphate (ADP), adenosine triphosphate (ATP) and guanosine triphosphate (GTP). Small amounts of inosine 5 monophosphate (IMP) and guanosine-diphosphate (GDP) (about 1 μ mole/l blood) were sometimes found in the controls but were never found in the patients. GMP, inosine-diphosphate (IDP) and inosine triphosphate (ITP) were never found in detectable amounts (\geq 1 μ mole/l blood).

The normal values found for AMP, ADP, ATP and GTP agree with the values reported

Because these nucleotides are water soluble the concentration per litre of free cell water is the best mode of expression giving the most information of the concentration of compounds involved in biochemical reactions (7).

Before adenine administration in both patients we found a decreased concentration of AMP, ADP and ATP and a normal concentration of GTP.

During oral administration of adenine normal values for AMP, ADP and ATP were found. GTP concentration was found normal or slightly increased.

DISCUSSION

Several investigators searched for the cause of increased purine synthesis *de novo* in Lesch Nyhan syndrome. Rosenbloom et al (16) discussed the following suppositions:

1 The decreased resynthesis of GMP and IMP results in a higher availability of phosphoribosylpyrophosphate (PRPP) for the synthesis of phosphoribosylamine (Fig. 1).

2 In HGPRTase deficient fibroblasts PRPP amidotransferase is activated by an increased concentration of hypoxanthine and guanine which are found to be inhibitors in normal cells (16).

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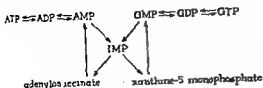


Fig 2 Interconversion of purine nucleotides

reactions in a cyclic interconversion of adenine and hypoxanthine derivatives and that a deficiency of this enzyme results in a depletion of intracellular adenine nucleotides

Wijngaarden & Ashton (19) investigated the influence of the various purine nucleotides on the activity of PRPP amidotransferase obtained from pigeon liver. ATP and ADP had the strongest inhibiting effect followed by GMP, AMP, IMP and GDP.

Henderson (6) found in Ehrlich ascites tumor cells a more potent inhibition by adenine than by guanine and showed that the purine bases have to be readily converted to ribonucleotides in order to exert their inhibiting effect.

It is highly probable that in Lesch Nyhan patients the increased purine synthesis *de novo* is caused by the decreased concentration of adenine nucleotides.

A more detailed study has to be made on the feedback inhibition of the purine synthesis *de novo* in human tissue.

If a real equilibrium exists between the three monophosphoribonucleotides AMP, IMP and GMP (Fig 2) this balance must be disturbed in Lesch Nyhan syndrome causing a selective decrease of AMP resulting in a much larger decrease of ADP and ATP.

SUMMARY

This paper presents the results of an investigation on the concentration of purine nucleotides in erythrocytes of two children with the Lesch Nyhan syndrome before and during oral adenine administration. The nucleotides AMP, ADP, ATP and GTP could be calculated. Before administration the patients showed a decreased concentration of the three adenine

nucleotides expressed as μ moles per litre of free cell water. The concentration of GTP was found to be normal. After oral adenine administration adenine nucleotides were found to be normal and GTP showed a slight increase. Some suppositions about the mechanism of increased purine synthesis *de novo* in patients with the Lesch Nyhan syndrome have been discussed.

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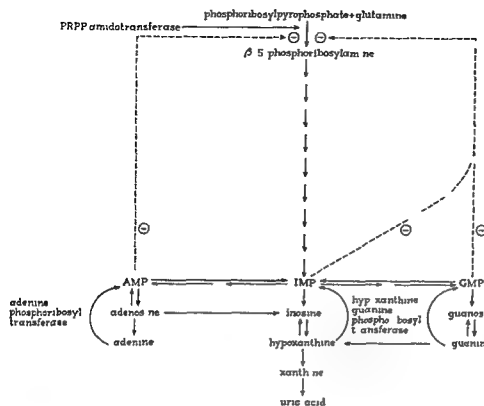


Fig 1 Purine synthesis *de novo* and purine metabolism

3 A decreased feedback inhibition of purine synthesis *de novo* caused by a decreased concentration of GMP and IMP. Rosenbloom examined the purine nucleotides content of normal fibroblasts and those of patients with the Lesch-Nyhan syndrome, and found no significant difference between both kinds of cells. These fibroblasts were cultured in Eagle medium containing a large amount of glutamine which is a precursor in purine synthesis *de novo*.

Ghadimi et al (5) concluded from the study of Seegmüller et al (17) that HGPRTase deficient cells contain an increased amount of AMP. However, this conclusion is based on an erroneous interpretation of the results of Seegmüller and co-workers who only demonstrated that adeninephosphoribosyltransferase (APRTase) activity is higher in HGPRTase deficient red cells than in normal cells.

Manzke (12) and Labrune et al (10) determined the concentrations per litre of erythrocytes of AMP, ADP and ATP in patients with the Lesch-Nyhan syndrome. Manzke found normal values but calls his methods in question himself. Labrune and co-workers found in-

creased values for adenine nucleotides but the authors did not mention their methods of determination. Furthermore, these determinations are performed a few days after a blood transfusion.

The present investigation demonstrates that erythrocytes from patients with the Lesch-Nyhan syndrome contain a decreased amount of adenine nucleotides, whereas the GTP content is normal. After oral administration of adenine, the adenine nucleotides were normalized and GTP showed a slight increase. Previously (20) we showed that purine synthesis *de novo* in Lesch-Nyhan patients was reduced during oral adenine administration as compared to the untreated patients. The results focus our attention to the role of adenine nucleotides, since the concentration of GTP was found to be normal in the patients. After administration of adenine, the decrease of purine synthesis *de novo* could be caused by an increase of feedback inhibition by adenine nucleotides.

The decrease of adenine nucleotides in our untreated patients can be explained according to Balis (2) and Sørensen (18) who postulated that HGPRTase completes the sequence of

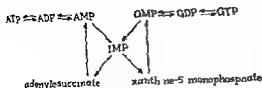


Fig 1 The conversion of purine nucleotides

reactions in a cyclic interconversion of adenine and hypoxanthine derivatives and that a deficiency of this enzyme results in a depletion of intracellular adenine nucleotides

Wijnjaarden & Ashton (19) investigated the influence of the various purine nucleotides on the activity of PRPP amidotransferase obtained from pigeon liver. ATP and ADP had the strongest inhibiting effect followed by GMP, AMP, IMP and GDP.

Henderson (6) found in Ehrlich ascites tumour cells a more potent inhibition by adenine than by guanine and showed that the purine bases have to be readily converted to ribonucleotides in order to exert their inhibiting effect.

It is highly probable that in Lesch Nyhan patients the increased purine synthesis *de novo* is caused by the decreased concentration of adenine nucleotides.

A more detailed study has to be made on the feedback inhibition of the purine synthesis *de novo* in human tissue.

If a real equilibrium exists between the three monophosphoribonucleotides AMP, IMP and GMP (Fig 2) this balance must be disturbed in Lesch Nyhan syndrome causing a selective decrease of AMP resulting in a much larger decrease of ADP and ATP.

SUMMARY

This paper presents the results of an investigation on the concentration of purine nucleotides in erythrocytes of two children with the Lesch Nyhan syndrome before and during oral adenine administration. The nucleotides AMP, ADP, ATP and GTP could be calculated. Before administration the patients showed a decreased concentration of the three adenine

nucleotides expressed as μ moles per litre of free cell water. The concentration of GTP was found to be normal. After oral adenine administration adenine nucleotides were found to be normal and GTP showed a slight increase. Some suppositions about the mechanism of increased purine synthesis *de novo* in patients with the Lesch Nyhan syndrome have been discussed.

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ANAPHYLAXIS AND RED CELL SURVIVAL STUDIES IN A CHILD WITH INSULIN RESISTANT DIABETES MELLITUS

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A syndrome of lymphadenopathy hepatosplenomegaly Coombs positive hemolytic anemia and leukopenia in an insulin resistant juvenile diabetic have previously been described by us (8-9). Immunological investigations showed that the child had high or normal immunoglobulin levels but that he was immunologically deficient as manifest by poor humoral antibody responses and defective cellular immunity (9). Clinical studies of the patient's serum revealed immune complexes of insulin anti insulin specificity and similar investigations in other diabetics have shown that such antigen antibody complexes are not uncommon in diabetic sera (4-14-15).

Immune complexes are known to lyse cells (11-20) and mediate anaphylactic reactions (12-13). It was therefore deemed important to study the patterns of erythrocyte sequestration and red cell survival in a diabetic known to have antigen antibody complexes in the circulation. Also this was an opportunity to study the anaphylactic properties of the patient's serum. The results showed that red cell survival is dependent on the type of insulin employed during therapy and that the anaphylactic properties of the antibody is of prognostic value in predicting the patient's response to corticosteroid therapy.

MATERIALS AND METHODS

Red cell survival and sequestration studies were done with Cr labelled erythrocytes. All survival curves were extended over 2 weeks to obtain accurate decay data and sequestration data was obtained by counting over the heart, spleen and liver. Passive cutaneous anaphylaxis (PCA) was done in guinea pigs (18) and rabbits (6) and vascular permeability was monitored with Evans blue (18) and an isotope tracer (7). Immune complexes were qualitatively identified by means of autoradiography (4-21) and were quantitatively measured according to a dialysis technique (14-15). Anti insulin antibodies were measured by the hydrodynamic flow method (10). IgG was prepared from the patient's serum by means of ion-exchange chromatography on DEAE cellulose using a Tris-phosphate continuous gradient elution scheme (5). Complement fixation was studied by means of passive immune lysis (19).

CASE REPORT

The patient is a 10 year old white male who was diagnosed as having diabetes mellitus in February 1963. In April 1963 he presented in ketoacidotic coma and was hospitalized when he was found to have hepatosplenomegaly and lymphadenopathy. His anti insulin titer was 12 (normal <1). He was discharged on a regimen of 30 mg of prednisone and 170 units of pork insulin per day and the prednisone was discontinued in May 1964. In 1965 the patient was found to be hypergammaglobulinemic with normal IgA and IgM but elevated IgG (24 mg/ml normal 10 mg/ml). In 1966 the IgG was 13.5 mg/ml and he was receiving 75 units of pork insulin per day. Lymphadenopathy and splenomegaly were recorded in February 1967. Serum IgG was elevated



Fig 1 Autoradiograph of anti insulin activity before and after immune complex dissociation. Whole serum on left and dissociated serum on right. Patient's serum in the well and the trough contained 1 μ insulin followed by rabbit anti human coprecipitating antibody

(14 mg/ml) but the insulin requirements had fallen to 54 units (pork) per day. In August 1967 the patient was found to be pale and his hemogram revealed 6 g/100 ml of hemoglobin (Hgb) hematocrit (Hct) 18% normal platelets and a reticulocyte count of 10.6%. The leukocyte count (WBC) was 1 850/mm³ with 20% neutrophils 72% lymphocytes 5% monocytes and 3% basophils. The direct Coombs test was strongly positive. A diagnosis of Coombs positive hemolytic anemia was established and an erythrocyte sequestration study was done before the institution of therapy. Then a regimen of 40 mg

prednisone and 63 units of pork insulin per day was begun. Hemolysis ceased and the lymph nodes and spleen returned to normal. The prednisone was stopped in January 1968 at which time the hemogram showed 13.2 g/100 ml Hgb 40% Hct and WBC 12 450 with 90% neutrophils and 10% lymphocytes. The anti insulin titer was 9, the serum IgG was 7.2 mg/ml and the insulin requirements had fallen to 45 units (pork) per day. In April 1968 the patient again presented with hepatosplenomegaly and lymphadenopathy and a hemogram showed 8.5 g/100 ml Hgb 25% Hct normal platelets and a WBC of 3 500. Prednisone was begun at 80 mg/day for 1 month. It was then changed to 60 mg every other day for 2 weeks and was stabilized at 40 mg every other day. In July 1968 the patient's hemogram, lymph nodes, liver and spleen were normal. In 1969 a red cell survival study was performed while he was receiving either pork or de-alaninated pork insulin. He presently receives 71 units of pork insulin per day and 40 mg of prednisone every other day. He has grown and done well in school and is presently in the 50 percentile for height and weight.

It has been repeatedly observed that the patient's insulin requirements have increased when he has received prednisone. The steroid has unquestionably controlled his hemolytic anemia and diminished the degree of hepatosplenomegaly and lymphadenopathy. However his physicians have been regularly met with a 20-25% increase in the patient's insulin need during corticosteroid therapy. Multiple marrow aspiration and lymph node biopsies have not supported a diagnosis of malignancy of the blood or blood forming organs. Immunopathological, immunohematological and immunochemical studies of this case have been published (9).

RESULTS

Demonstration of immune complexes

This has been extensively studied and reported in references (4, 8, 9, 14, 15). The serum used for the anaphylaxis experiments had an anti insulin titer of 9 before antigen antibody dissociation and a titer of 27 after dissociation. The post dissociation sample showed a longer and more dense antibody arc in the radioautograph than the pre dissociation sample as seen in Fig 1. Undiluted patient's serum (0.1 ml) injected intradermally into a blue guinea pig (3) caused a bluing reaction of 2+ in intensity and 11 mm diameter. Other diabetic sera used in the anaphylaxis assay were negative when diluted 1:10 in the blue guinea pig test. Undiluted patient's serum provoked a



Fig 2 Passive cutaneous anaphylaxis. From Table 1 7, 8 and 9 are non-diabetic serum (adult), non-diabetic serum (neonatal) and patient's IgG respectively. Numbers 1, 2 and 3 are the prednisone responsive, insulin resistant adult and child diabetic sera, and 4, 5 and 6 are 1/10 dilutions of 1, 2 and 3. Note that the patient is negative.

complement mediated passive immune lysis of sheep erythrocytes, but the patient's IgG was negative in this test.

Anaphylaxis experiments

PCA studies were done with an IgG preparation from the patient's serum. Sera from three prednisone responsive insulin resistant patients were used as positive controls and two non-diabetic sera were used as negative controls. Guinea pigs were intradermally sensitized with 1/10 dilutions of the test and control sera and insulin Evans blue and 2 μ Ci of I^{125} labelled guinea pig IgG were given intravenously 4 hours later. The negative controls and the patient's IgG were negative, but the

positive controls showed qualitatively positive reactions as seen in Fig 2. These data are quantitatively expressed in Table 1 and they clearly show that the patient's antibody is negative in spite of its high titer of anti-insulin activity. The patient's IgG was again negative when studied in rabbits employing a 24 hour latent period (6).

Sequestration and survival of erythrocytes

Radiobiological experiments were done in 1967 to monitor the sites of red cell sequestration and in 1968 to determine the *in vivo* survival time for erythrocytes. These experiments were done after the onset of hemolytic anemia.

Table 1 *Passive cutaneous anaphylaxis as an index of prednisone responsiveness*

Samples	Anti insulin titer (10)	Passive cutaneous anaphylaxis		
		Intensity (18)	Millimeters (18)	Counts/Min (7)
Non-diabetic adult serum	0	0	0	52
Non-diabetic neonatal serum	0	0	0	41
Prednisone-responsive insulin resistant adult diabetic sera	8-5	2+	10	671
Prednisone responsive insulin resistant diabetic serum, child	13	3+	18	1180
Patient's IgG	15-9*	3+ 0	18 0	1496 67

Sera known to contain anti-insulin antibodies of IgG specificity (4)

Table 2 ^{51}Cr RBC sequestration data

Day of study	Body counts			Spleen/Liver Ratio
	Heart	Spleen	Liver	
1	1 940	3 874	1 964	2.5:1
2	1 919	3 452	1 497	2.3:1
5	1 367	3 103	1 163	2.7:1

(a) Red cell sequestration This investigation was done before institution of prednisone therapy, and results were compatible with a diagnosis of hypersplenism. Counts over the heart, spleen, and liver for 5 days revealed a 2.7:1.0 ratio for spleen/liver as seen in Table 2.

(b) Red cell survival Experiments were done after the diagnosis and control of the hemolytic process in order to study the relationship of the administered insulin to the patient's hemolysis. Baseline determinations of red cell survival were done while the patient received pork insulin and prednisone. A hypoallergenic insulin was then substituted for his usual insulin. The modified product was pork insulin from which the C terminal amino acid alanine had been removed (1, 2). Erythrocyte survival studies were repeated after the patient had been receiving the dealaninated insulin for 1 month. The data in Table 3 show that the erythrocytes experienced a longer survival while the patient was receiving his usual insulin than when he received the dealaninated insulin. Body counts at this time revealed no splenic sequestration of red cells.

Table 3 Erythrocyte survival data

Date	Insulin therapy	Label	RBC survival	
			Patient	Normal
Jan 1969	Pork	$99\ \mu\text{Ci}\ ^{51}\text{Cr}$	23 days	30 ± 3 days
March	Dealaninated pork		16 days	30 ± 3 days

DISCUSSION

The PCA experiments produced positive reactions with several sera from prednisone responsive insulin resistant diabetics, but serum from the patient under study was consistently negative (Fig. 2 and Table 1). Prednisone responsive diabetics are insulin resistant patients who require less insulin while under going steroid therapy (16). The patient under study required more insulin while receiving prednisone, and his anti insulin antibody was PCA negative. These data are consistent with the Oakley hypothesis that sera from insulin-resistant diabetics who require less insulin during prednisone therapy demonstrate positive PCA reactions, and insulin resistant diabetics requiring more insulin during prednisone therapy give negative PCA reactions (17). The test is a useful prognostic adjunct in determining whether or not an insulin resistant diabetic is going to respond to prednisone.

The hemolytic process was controlled with oral prednisone therapy, but the underlying pathophysiology was only suppressed and not corrected. Adequate suppression was demonstrated in the red cell sequestration studies where splenic trapping was demonstrated before but not after corticosteroid therapy (Table 2). The reappearance of lymphadenopathy and hemolysis with efforts to discontinue prednisone are corroborative clinical evidence that the basic disease was dampened but not eliminated by steroids.

The red cell survival experiments done while the patient was receiving pork and dealaninated insulin was used. The substitution of dealaninated insulin for the patient's usual insulin created a situation in which immune globulins with specificity for the C terminal amino acid of pork insulin could no longer combine with their antigen *in vivo*. The net effect of this change was an elevation in the anti insulin titer. This conclusion is strengthened by the fact that we have now documented similarly elevated anti insulin titer following insulin changes while cycling another diabetic through beef, pork and fish insulins.

(14-15) Since the patient's hemolytic anemia is causally related to his anti-insulin antibodies (9) it is not surprising that his red cell survival decreased when the insulin type was changed (Table 3). This represents the first demonstration that erythrocyte survival in diabetes is dependent on the type of insulin employed during therapy.

SUMMARY

A child with the syndrome of diabetes mellitus, insulin resistance, Coombs positive autoimmune hemolytic anemia, lymphadenopathy and hepatosplenomegaly was described. The patient's IgG anti-insulin antibody was shown to be negative for passive cutaneous anaphylaxis (PCA). Inasmuch as the patient was refractory to prednisone therapy and presented a negative PCA test, he was consistent with the Oakley hypothesis that insulin-resistant prednisone-insensitive patients will be negative for PCA. The half-life of the patient's red blood cells was shown to vary as a function of the type of insulin used in therapy. The child was shown to have immune-complexes in his serum of insulin anti-insulin specificity, and it was suggested that these complexes are partially responsible for the microangiopathy that is often observed in diabetes mellitus.

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AN EPIDEMIOLOGICAL STUDY OF CHILD HEALTH AND NUTRITION IN A NORTHERN SWEDISH COUNTY

III Medical and anthropometrical examinations

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In recent years fairly extensive epidemiological studies of the health status of children in Sweden have been published by Mellander et al (15) Mellbin (16) Sterky (22) and Karlberg et al (11). These studies have mainly been concerned with special groups in the population and with infants and children up to three years of age.

The aim of this investigation was to study the health and nutritional status of three age groups of children in different parts of a northern Swedish county at the same time that an investigation of food intake was conducted (18) and to relate the findings to food habits and socio-economic factors.

MATERIAL

All of the 1401 children investigated were selected from three areas of Västerbotten county which differ from one another geographically and in part socio-economically, namely one urban area and two rural areas (Fig 1). All children were selected from the population register for Västerbotten county. A detailed description of the selection procedure is given by Samuelson 1971 (18). Three age groups were studied: 4-, 8- and 13-year-olds with exact decimal median ages of 4.378 years, 8.296 years and 13.317 years. In all three areas 8- and 13-year-olds were studied, while in the city of Umeå also 4-year-olds were included. The composition of the material and its age and sex distribution is shown in Table 1. The reasons for the omission of certain children in Table 2.

All urban children were from the city of Umeå near the coast of the Gulf of Bothnia. Every second child in the age groups, 4-, 8- and 13 years was selected from

the population register for the parts of the city of Umeå proper.

In the inland area which is rural all children in the 8- and 13-year-old groups were included. For practical reasons the study included only school children in this area.

In the third area the mountain foreland as in the inland area all 8- and 13-year-olds were included.

Details about the geography and population are given by Samuelson 1971 (18).

METHODS

The medical examination

All children underwent a medical examination. All the examinations were performed during the autumn of 1967.

The same paediatrician (the author) examined all the children in the study and personally performed all the measurements and assessments. The preschool children were examined in a child health centre in the city of Umeå. All the school children were examined at school under comparable conditions in the three areas. The same equipment was used throughout the study. The medical examination of a child took an average of 10-15 min.

1 The physical examination

The physical examination was performed according to a previously determined scheme. The children's general condition was not graded. The mouth, lips and throat were examined and angular lesions were recorded. Conjunctivitis or other changes in the conjunctivae were noted. Upper respiratory infections such as pharyngitis and signs of the common cold were recorded. The teeth and gums were not included in this examination, but a special odontological examination was performed by a dentist immediately before or after the medical examination. This part of the study is described by Samuelson et al (19).

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Umeå General Hospital according to an immunochemical method described by Laurell (13)

Anthropometrical measurements

All the measurements on a given child were performed on the same occasion. Internationally accepted techniques were used (3 7 10 24)

Weight

All children were weighed on the same platform balance (Stathmos 304 AB Lindell Jonköping Sweden). This balance is robust and easy to transport. It was protected from mechanical damage in transport by a 4 cm thick sheetrubber carpet. Before use the zero setting of the balance was checked with 10 and 20 kg weights and this check was repeated 4-5 times each day. Control weighings of the weights at the National Commission of Standards showed that they differed by no more than +3 g from 10 and 0 kg respectively.

During the investigation the balance was checked each month at the workshop of Umeå University Hospital.

The children were weighed naked or wearing only light underwear. They stood on the centre of the platform. All weighings were done by the same person who recorded the weights to the nearest 0.1 kg.

Height

For measuring the 4 year olds a scale fixed to a wall was used. The child stood barefoot on the floor with the feet parallel and the heels buttocks and back of the head touching the wall. The arms were at the sides. A wooden headpiece was lowered until it touched the top of the head. The measurement was recorded to within 0.5 cm.

The school children were measured using a vertical metal measuring rod attached to the platform balance used for weighing. The children stood on the platform with the feet parallel and with the heels at the end of the platform in the same position for each child. The buttocks shoulders and back of the head were in contact with the rod and the arms were at the sides. The top of the metal rod bent 90° was lowered until it touched the top of the head. The measurements were recorded to within 0.5 cm. The measuring scale was regularly checked by comparing the result of measuring a child with this scale with that obtained using a scale fixed to the wall. All measurements were carried out by the same person.

Subcutaneous fat

For determination of skinfold thickness a skinfold caliper (model Harpenden British Indicators Limited) was used. The determinations were performed by the same person using the same caliper for all children. The Harpenden caliper exerted a constant pressure of 10 g/mm. By interpolation between the markings on the dial thickness was measured to the nearest 0.1 mm.

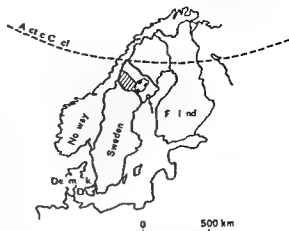


Fig 1 A map of Scandinavia showing the county of Västerbotten and the three areas studied. The arrow indicates the city of Umeå, the stippled area the inland area, the shaded area the mountain foreland.

The triceps skinfold was measured in the middle part of the left arm at a line between the tip of the acromion process of the scapula and the olecranon process of the ulna. The longitudinal skinfold and the subcutaneous tissue was picked up between the thumb and forefinger of the left hand drawn away from the underlying muscle and measured. Three measurements were taken and the mean value of these was recorded.

The subscapular skinfold was measured just below and lateral to the angle of the left scapula. A skinfold was picked up parallel to the natural cleavage line of the skin. Three measurements were taken and the mean value of these was recorded.

For both triceps and subscapular skinfolds the error of a single determination was calculated from 100 duplicate determinations in each age group and was found to be ± 0.1 mm for all age groups.

Data on ESR are lacking for 3% of the 8 year-olds and 2% of the 13 year-olds. Serum albumin levels are lacking for 17% of the 4 year-olds, 23% of the 8 year-olds and 20% of the 13 year-olds. Values for these children are missing because of technical difficulties, for example hemolysis in the serum or the destruction of micro-capillary tubes in transport. In the 4 year-old group data on skinfold thickness are lacking for 24% of the children. The measurements on these 47 children were not included because initial difficulty in repeatedly measuring the skinfold led to uncertain values.

Statistical methods

The methods used are described elsewhere (18).

According to $s = \pm \sqrt{Sd^2/n}$ where d is the difference between duplicate determinations and n is the number of the differences.

Table 1 Age, sex and geographical distribution of the 1401 children studied

Age group Median age	4-year olds 4 years 5 months			8 year olds 8 years 4 months			13 year olds 13 years 4 months		
	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
City of Umeå	99	99	198	100	100	200	100	100	200
Inland area	—	—	—	98	90	188	91	111	202
Mountain foreland	—	—	—	99	96	195	114	104	218
Total	99	99	198	297	286	583	305	315	620

Pathological findings in the superficial lymph nodes in the neck and in the axillae were recorded. The thyroid gland was examined for enlargement.

Dermatoses and allergic and infectious manifestations in the skin were noted. Unusual properties of the hair such as thinness and dyspigmentation were looked for. The nails were examined for brittleness.

The blood pressure and the heart rate were not registered but pathological auscultation findings in the heart and lungs were recorded.

The abdomen was palpated for tumors or possible enlargement of the liver or spleen.

The muscle mass was not recorded but signs of muscular dystrophy or wasting were looked for.

The genital organs of boys were examined for undescended testes.

Abnormal findings in the skeletal system such as deformities, Harrison's sulcus of the chest and other

aberrations were looked for. The general gait was observed in all children.

2 Microsedimentation rate (ESR)

ESR was determined according to Strom (23). Within two hours of blood sampling the mixture of blood and anticoagulant was drawn into micropipettes for the determination. The room temperature varied between 18 and 22°C.

3 Albumin concentration

Albumin was determined on serum from capillary blood samples. The samples were drawn from finger pricks into heparinized capillary tubes and were centrifuged in an International Micro-Capillary Centrifuge. The centrifugation time was 4-5 min and the speed 11 000 rpm. The albumin determinations were performed at the Department of Clinical Chemistry.

Table 2 Distribution of omissions in the different areas

1 = City of Umeå 2 = Inland area 3 = Mountain foreland

	1	2	3	Total	Per cent
Invited	635	411	442	1488	100.0
Moved	29 ^a	12	18	59	4.0
Remained to examine	606	399	424	1429	96.0
Non Caucasian (age uncertain)	1	—	—	1	0.1
Mental retardation	5	3	6	14	1.0
Blindness (cerebromacular degeneration)	—	—	1	1	0.1
Diabetes mellitus	—	3	1	4	0.3
Chondrodystrophy	—	1	—	1	0.1
Remained to examine	600	392	416	1408	94.6
Refused	—	—	2	2	0.1
Excluded because food consumption at recall was incomplete due to	—	—	—	—	—
Acute gastroenteritis	2	—	1	3	0.2
Upper respiratory infection	—	2	—	2	0.1
Final material taking part in the investigation	598	390	413	1401	94.2

^a Of these fifteen were 4 year olds.

Imo General Hospital according to an immunological method described by Laurell (13)

Anthropometrical measurements

The measurements on a given child were performed on the same occasion. Internationally accepted angles were used (3, 7, 10, 24)

Weight

Children were weighed on the same platform scale (Stathmos 304 AB Lindell Jonköping Sweden). This balance is robust and easy to transport and is protected from mechanical damage in transit by a 4 cm thick sheetrubber carpet. Before use zero setting of the balance was checked with 10, 0 kg weights and this check was repeated 4-5 times each day. Control weighings of the weights at the National Commission of Standards showed that they differed by no more than +3 g from 10 and 0 kg respectively.

During the investigation the balance was checked 1 month at the workshop of Umeå University Hospital.

The children were weighed naked or wearing only underwear. They stood on the centre of the platform. All weighings were done by the same person and recorded to the nearest 0.1 kg.

Height

For measuring the 4 year-olds a scale fixed to a wall was used. The child stood barefoot on the floor with feet parallel and the heels, buttocks and back of head touching the wall. The arms were at the sides. A wooden headpiece was lowered until it touched the top of the head. The measurement was recorded to within 0.5 cm.

For school children were measured using a vertical measuring rod attached to the platform balance for weighing. The children stood on the platform with the feet parallel and with the heels at the back of the platform in the same position for each child. The buttocks, shoulders and back of the head were in contact with the rod and the arms were at the sides. The top of the metal rod bent 90° was used until it touched the top of the head. The measurements were recorded to within 0.5 cm. The measuring scale was regularly checked by comparing the result of measuring a child with this scale with that obtained using a scale fixed to the wall. All measurements were carried out by the same person.

Subcutaneous fat

For determination of skinfold thickness a skinfold thickness gauge (model Harpenden British Indicators Limited) was used. The determinations were performed by the same person, using the same caliper for all children. Harpenden caliper exerted a constant pressure of 10 g/mm². By interpolation between the markings on the dial thickness was measured to the nearest 0.1 mm.

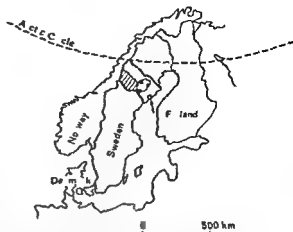


Fig. 1 A map of Scandinavia showing the county of Västerbotten and the three areas studied. The arrow indicates the city of Umeå, the stippled area the inland area, the shaded area the mountain foreland.

The triceps skinfold was measured in the middle part of the left arm at a line between the tip of the acromion process of the scapula and the olecranon process of the ulna. The longitudinal skinfold and the subcutaneous tissue was picked up between the thumb and forefinger of the left hand drawn away from the underlying muscle and measured. Three measurements were taken and the mean value of these was recorded.

The subscapular skinfold was measured just below and lateral to the angle of the left scapula. A skinfold was picked up parallel to the natural cleavage line of the skin. Three measurements were taken and the mean value of these was recorded.

For both triceps and subscapular skinfolds the error of a single determination was calculated from 100 duplicate determinations in each age group and was found to be ± 0.1 mm for all age groups.

Data on ESR are lacking for 3 of the 8 year olds and 2% of the 13 year-olds. Serum albumin levels are lacking for 17 of the 4 year olds, 23 of the 8 year olds and 20% of the 13 year olds. Values for these children are missing because of technical difficulties for example hemolysis in the serum or the destruction of micro-capillary tubes in transport. In the 4 year old group data on skinfold thickness are lacking for 24% of the children. The measurements on these 47 children were not included because initial difficulty in repeatedly measuring the skinfold led to uncertain values.

Statistical methods

The methods used are described elsewhere (18).

According to $\sigma = \sqrt{\sum d^2 / 2n}$ where d is the difference between duplicate determinations and n is the number of the differences.

Table 3 Summary of clinical findings in the examination

Diagnosis	4 year olds		8 year olds		13 year olds	
	No		No		No	
Upper respiratory infection	22	11.1	28	4.8	38	6.1
Bronchitis	2	1.0	5	0.9	1	0.2
Acute conjunctivitis	1	0.5	2	0.3	0	—
Angular lesions	0	—	5	0.9	2	0.3
Enlargement of the thyroid gland	0	—	0	—	9	1.5
Eczema	12	6.1	3	0.5	13	2.1
Impetigo	0	—	3	0.5	5	0.8
Valvular aortic stenosis	0	—	0	—	1	0.2
Ventricular septal defect	0	—	0	—	1	0.2
Inguinal hernia	0	—	2	0.3	0	—
Hydrocele testis	1	1.0	0	—	0	—
Undescended testis	2	2.0	4	1.3	2	0.7
Hypospadias	0	—	1	0.2	1	0.2
Minor cerebral palsy	0	—	1	0.2	2	0.3

RESULTS

I Medical examination

The results of the medical examination are shown in Table 3

4 year old group The general nutritional status was good for all children in the group. Upper respiratory infections (rhino naso-pharyngitis and/or enlarged and tender superficial lymph nodes in the neck) were noted in 11.1% at the time of examination. Mild eczema, mainly in the bend of the arm and on the cheeks, was present in 6.1%. At the time of the examination, no child had severe or weeping eczema.

Physiological heart murmurs were registered but have not been included in the summary of the findings. No hepatic or splenic enlargement was found.

8 year-old group The general nutritional status was good for all children. Upper respiratory infections were noted in 4.8% of the children at the time of examination. There were dry eczematous skin changes in 0.5% of the children.

No hepatic or splenic enlargement was found.

One child had partial hemiparesis due to birth injury, but the damage was not so severe as to constitute a handicap.

Comparison between the different areas re-

vealed no differences in general health status. The frequency of upper respiratory infections and eczema was about the same in all areas.

13-year-old group The general nutritional status was good. Upper respiratory infections were present in 6.1% of the children at the time of examination. Thyroid enlargement was found in 1.5% of the 13-year olds, all girls. Skin changes of an eczematous type were present in 2.1% of the children. Auscultation of the heart revealed that two children had systolic murmurs of a pathological type. Previous investigation at the Department of Paediatrics, Umeå, had established that one of these children had subvalvular aortic stenosis and the other had a ventricular septal defect. Both children were free of subjective symptoms.

No hepatic or splenic enlargement was found. Two children had inguinal hernias.

Two children had non handicapping residual neurological damage resembling mild cerebral palsy.

One child was found to have moderate icterus. Later investigation showed that this was familial, non hemolytic icterus (Gilbert's disease).

Comparison between the different areas revealed no differences in the general health status and no differences in the frequency of upper

Table 4 Mean values and standard deviations (SD) of ESR in mm

	4-year-olds			8 year-olds			13-year-olds		
	No	Mean	SD	No	Mean	SD	No	Mean	SD
Boys	97	18	12	289	13	10	297	13	11
Girls	95	21	12	275	14	9	311	15	9
Total	192	20	12	564	14	10	608	14	9

respiratory infections. The frequency of eczema was the same in all areas. Thyroid enlargement was found only in 13 year-old girls in the inland area (5 cases) and in the mountain foreland (4 cases).

II. Microsedimentation rate (ESR) and serum albumin

4 year old group There was no significant sex difference in the mean value of the ESR (Table 4). Of the 4-year-olds 38% had an ESR above 20 mm. The mean value for the ESR in this age group was significantly higher than the corresponding mean values in the 8- and 13-year-old groups ($p < 0.01$).

The means and standard deviations for serum albumin levels are given in Table 5.

8 year-old group There was no significant sex difference in the mean value of the ESR (Table 4).

On comparison between the different areas it appeared that 16% of the children in the city of Umeå, 12% in the inland area and 21% in the mountain foreland had ESR values above 20 mm. The children in the mountain foreland had a higher mean ESR value than the children in the inland area ($p < 0.05$).

There were no significant differences in the serum albumin levels either between the sexes or between the different areas (Table 5).

13 year-old group As a group the girls had a somewhat higher mean ESR than the boys ($p < 0.01$).

Comparison between the different areas revealed no significant differences in the mean ESR values. ESR values above 20 mm were found in 18% of the children in the city of Umeå, 19% in the inland area and 21% in the mountain foreland.

The mean value for serum albumin which is shown for the group as a whole in Table 5 did not differ between the sexes or between the different areas.

III. Anthropometrical measurements

Table 6a-d shows the means and standard deviations and Fig 2a-d the percentiles for weight, height and triceps and subscapular skin fold thickness.

4 year-old group There were no significant sex differences in weight and height. On the other hand the mean values for triceps and subscapular skinfold thickness were higher in girls than in boys ($p < 0.01$).

Table 5 Mean values and standard deviations (SD) of albumin in serum of 4, 8- and 13-year-old children

The values are given in g/100 ml

	4-year-olds			8 year-olds			13-year-olds		
	No	Mean	SD	No	Mean	SD	No	Mean	SD
Boys	83	4.34	0.40	216	4.42	0.39	238	4.56	0.38
Girls	81	4.31	0.38	231	4.52	0.42	256	4.51	0.34
Total	164	4.33	0.39	447	4.47	0.41	494	4.54	0.36

Table 3 *Summary of clinical findings in the examination*

Diagnosis	4 year olds		8 year olds		13 year olds	
	No		No		No	
Upper respiratory infection	22	11.1	28	4.8	38	6.1
Bronchitis	2	1.0	5	0.9	1	0.2
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Angular lesions	0	—	5	0.9	2	0.3
Enlargement of the thyroid gland	0	—	0	—	9	1.5
Eczema	12	6.1	3	0.5	13	2.1
Impetigo	0	—	3	0.5	5	0.8
Valvular aortic stenosis	0	—	0	—	1	0.2
Ventricular septal defect	0	—	0	—	1	0.2
Inguinal hernia	0	—	2	0.3	0	—
Hydrocele testis	1	1.0	0	—	0	—
Undescended testis	2	2.0	4	1.3	2	0.7
Hypospadias	0	—	1	0.2	1	0.2
Minor cerebral palsy	0	—	1	0.2	2	0.3

RESULTS

I Medical examination

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Physiological heart murmurs were registered but have not been included in the summary of the findings. No hepatic or splenic enlargement was found.

8 year old group The general nutritional status was good for all children. Upper respiratory infections were noted in 4.8% of the children at the time of examination. There were dry eczematous skin changes in 0.5% of the children.

No hepatic or splenic enlargement was found.

One child had partial hemiparesis due to birth injury, but the damage was not so severe as to constitute a handicap.

Comparison between the different areas re-

vealed no differences in general health status. The frequency of upper respiratory infections and eczema was about the same in all areas.

13-year-old group The general nutritional status was good. Upper respiratory infections were present in 6.1% of the children at the time of examination. Thyroid enlargement was found in 1.5% of the 13 year olds, all girls. Skin changes of an eczematous type were present in 2.1% of the children. Auscultation of the heart revealed that two children had systolic murmurs of a pathological type. Previous investigation at the Department of Paediatrics, Umeå, had established that one of these children had subvalvular aortic stenosis and the other had a ventricular septal defect. Both children were free of subjective symptoms.

No hepatic or splenic enlargement was found. Two children had inguinal hernias.

Two children had non handicapping residual neurological damage resembling mild cerebral palsy.

One child was found to have moderate icterus. Later investigation showed that this was familial non hemolytic icterus (Gilbert's disease).

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Table 4 Mean values and standard deviations (SD) of ESR in mm

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Boys	97	18	12	289	13	10	297	13	9
Girls	95	21	12	275	14	9	311	15	9
Total	192	20	12	564	14	10	608	14	9

respiratory infections. The frequency of eczema was the same in all areas. Thyroid enlargement was found only in 13 year old girls in the inland area (5 cases) and in the mountain foreland (4 cases).

II. Microsedimentation rate (ESR) and serum albumin

4 year-old group There was no significant sex difference in the mean value of the ESR (Table 4). Of the 4-year-olds 38% had an ESR above 20 mm. The mean value for the ESR in this group was significantly higher than the corresponding mean values in the 8- and 13 year old groups ($p < 0.01$).

The means and standard deviations for serum albumin levels are given in Table 5.

8 year-old group There was no significant sex difference in the mean value of the ESR (Table 4).

On comparison between the different areas it appeared that 16% of the children in the city of Umeå, 12% in the inland area and 21% in the mountain foreland had ESR values above 20 mm. The children in the mountain foreland had a higher mean ESR value than the children in the inland area ($p < 0.05$).

Table 5 Mean values and standard deviations (SD) of albumin in serum of 4, 8- and 13 year old children

The values are given in g/100 ml

	4-year-olds			8 year-olds			13 year-olds		
	No	Mean	SD	No	Mean	SD	No	Mean	SD
Boys	83	4.34	0.40	216	4.42	0.39	238	4.56	0.38
Girls	111	4.31	0.38	231	4.52	0.42	256	4.51	0.34
Total	164	4.33	0.39	477	4.47	0.41	494	4.54	0.36

There were no significant differences in the serum albumin levels either between the sexes or between the different areas (Table 5).

13 year-old group As a group the girls had a somewhat higher mean ESR than the boys ($p < 0.01$).

Comparison between the different areas revealed no significant differences in the mean ESR values. ESR values above 20 mm were found in 18% of the children in the city of Umeå, 19% in the inland area and 21% in the mountain foreland.

The mean value for serum albumin which is shown for the group as a whole in Table 5 did not differ between the sexes or between the different areas.

III. Anthropometrical measurements

Table 6a-d shows the means and standard deviations and Fig. 2a-d the percentiles for weight, height and triceps and subscapular skin fold thickness.

4 year-old group There were no significant sex differences in weight and height. On the other hand the mean values for triceps and subscapular skinfold thickness were higher in girls than in boys ($p < 0.01$).

Table 6a Total material Weight height and skinfold thickness (Mean values and S D) in 4, 8, and 13 year old children

	No	Weight kg		Height cm		No	Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D		Mean	S D	Mean	S D
<i>4 year olds</i>										
Boys	99	18.1	2.4	107.0	4.7	88	8.6 **	1.9	5.0 **	1.2
Girls	99	17.9	2.4	106.5	4.2	63	9.4	1.8	5.5	1.3
Total	198	18.0	2.4	106.8	4.5	151	8.9	1.9	5.2	1.2
<i>8 year olds</i>										
Boys	297	26.9	4.3	129.6	5.5	297	8.2 ***	2.6	5.1 ***	2.1
Girls	286	26.8	4.6	129.3	5.6	286	10.1	3.3	6.5	3.5
Total	583	26.8	4.5	129.5	5.5	583	9.1	3.1	5.8	2.9
<i>13 year olds</i>										
Boys	305	45.3 ***	8.9	157.0	8.2	305	8.9 ***	3.4	6.4 **	3.3
Girls	315	47.8	8.0	158.0	6.7	315	12.1	4.4	8.6	4.0
Total	620	46.6	8.5	157.5	7.5	620	10.5	4.4	7.5	3.8

** $p < 0.01$ *** $p < 0.001$

8 year-old group There were no significant sex differences in weight and height (Table 6a). However the girls had significantly higher mean values for skinfold thickness than the boys ($p < 0.001$).

Comparison between the different areas revealed the following

Weight The girls in the city of Umea weighed more ($p < 0.05$) than the girls in the mountain foreland (Table 6e).

Height The girls in the city of Umea were somewhat taller than the girls in the inland area or the mountain foreland ($p < 0.05$). The 8 year olds were significantly taller ($p < 0.01$) in

Table 6b City of Umea Weight, height and skinfold thickness (Mean values and S D) in 8 and 13 year old children

		Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
No		Mean	S D	Mean	S D	Mean	S D	Mean	S D
<i>8 year olds</i>									
Boys	100	26.9	4.1	130.2	5.3	8.6 ***	2.5	5.3 ***	1.9
Girls	100	27.4	4.6	130.4	5.6	11.0	3.4	7.2	4.0
Total	200	27.2	4.4	130.3	5.5	9.8	3.2	6.2	3.3
<i>13 year olds</i>									
Boys	100	46.0	9.4	157.6	8.2	9.4 ***	3.9	6.9 **	3.8
Girls	100	47.3	7.9	158.9	6.3	12.0	4.2	8.6	4.3
Total	200	46.7	8.7	158.2	7.4	10.7	4.2	7.8	4.1

** $p < 0.01$ *** $p < 0.001$

Table 6c Inland area Weight height and skinfold thickness (Mean values and S D) in 8 and 13 year-old children

Year-old children									
	No	Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
<i>8 year-olds</i>									
Boys	98	27.0	4.1	129.8	5.7	8.1 ^{**}	2.6	5.0 ^{**}	1.8
Girls	90	26.7	5.5	128.8	5.7	9.7	3.5	6.5	4.0
Total	188	26.9	4.8	129.3	5.7	8.8	3.2	5.7	3.2
<i>13 year-olds</i>									
Boys	91	46.0	9.0	157.6	8.5	8.9 ^{**}	4.2	6.5 ^{**}	3.6
Girls	111	48.5	8.4	158.2	6.7	12.5	4.9	9.0	4.0
Total	202	47.3	8.8	158.0	7.5	10.9	4.9	7.9	4.0

$p < 0.05$ $p < 0.01$ *** $p < 0.001$

the city of Umeå than in the mountain foreland (Table 6e)

Triceps skinfold Boys in the city of Umeå had a significantly higher mean value for triceps skinfold than the boys in the mountain foreland ($p < 0.05$). Girls in the city of Umeå had a higher mean value for triceps skinfold than girls in the inland area ($p < 0.01$) or the mountain foreland ($p < 0.001$) (Table 6e).

Subscapular skinfold This skinfold measurement was greater for girls in the city of Umeå than for girls in the mountain foreland ($p < 0.01$). The same significant difference was also found for the group as a whole (Table 6e).

13 year-old group The girls had higher mean values for body weight and skinfold thickness than the boys (Table 6a). There was no significant sex difference in height.

Comparison between the different areas revealed the following:

Weight There were no significant differences between the children in the different areas with respect to body weight.

Height The mean height for the entire 13 year-old group in the city of Umeå was higher ($p < 0.05$) than that for the group in the mountain foreland (Table 6e).

Triceps skinfold No significant differences

Table 6d Mountain foreland Weight height and skinfold thickness (Mean values and S D) in 8 and 13 year old children

	No	Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
<i>8 year-olds</i>									
Boys	99	26.8	4.7	129.0	5.4	7.8	2.5	5.1	2.5
Girls	96	26.1	3.7	128.5	5.3	9.4	2.8	5.8	1.8
Total	195	26.5	4.2	128.8	5.3	8.6	2.8	5.4	2.2
<i>13 year-olds</i>									
Boys	114	44.2	8.2	156.0	7.8	8.4	3.4	5.9	2.2
Girls	104	47.6	7.7	157.0	7.1	11.9	4.0	8.2	3.9
Total	218	45.8	8.2	156.5	7.5	10.1	4.1	7.0	3.3

$p < 0.05$ $p < 0.01$ *** $p < 0.001$

Table 6a Total material Weight, height and skinfold thickness (Mean values and S D) in 4, 8, and 13 year old children

	No	Weight kg		Height cm		No	Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D		Mean	S D	Mean	S D
<i>4 year olds</i>										
Boys	99	18.1	2.4	107.0	4.7	88	8.6 ..	1.9	5.0 ..	1.2
Girls	99	17.9	2.4	106.5	4.2	63	9.4	1.8	5.5	1.3
Total	198	18.0	2.4	106.8	4.5	151	8.9	1.9	5.2	1.2
<i>8 year olds</i>										
Boys	297	26.9	4.3	129.6	5.5	297	8.2	2.6	5.1	2.1
Girls	286	26.8	4.6	129.3	5.6	286	10.1	3.3	6.5	3.5
Total	583	26.8	4.5	129.5	5.5	583	9.1	3.1	5.8	2.9
<i>13 year olds</i>										
Boys	305	45.3	8.9	157.0	8.2	305	8.9	3.4	6.4	3.3
Girls	315	47.8	8.0	158.0	6.7	315	12.1	4.4	8.6	4.0
Total	620	46.6	8.5	157.5	7.5	620	10.5	4.4	7.5	3.8

** $p < 0.01$ *** $p < 0.001$

8 year-old group There were no significant sex differences in weight and height (Table 6a). However the girls had significantly higher mean values for skinfold thickness than the boys ($p < 0.001$).

Comparison between the different areas revealed the following:

Weight The girls in the city of Umeå weighed more ($p < 0.05$) than the girls in the mountain foreland (Table 6e).

Height The girls in the city of Umeå were somewhat taller than the girls in the inland area or the mountain foreland ($p < 0.05$). The 8 year olds were significantly taller ($p < 0.01$) in

Table 6b City of Umeå Weight, height and skinfold thickness (Mean values and S D) in 8 and 13 year old children

		Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
No		Mean	S D	Mean	S D	Mean	S D	Mean	S D
<i>8 year-olds</i>									
Boys	100	26.9	4.1	130.2	5.3	8.6 ***	2.5	5.3 ***	1.9
Girls	100	27.4	4.6	130.4	5.6	11.0	3.4	7.2	4.0
Total	200	27.2	4.4	130.3	5.5	9.8	3.2	6.2	3.3
<i>13 year olds</i>									
Boys	100	46.0	9.4	157.6	8.2	9.4 ***	3.9	6.9 **	3.8
Girls	100	47.3	7.9	158.9	6.3	12.0	4.2	8.6	4.3
Total	200	46.7	8.7	158.2	7.4	10.7	4.2	7.8	4.1

** $p < 0.01$, *** $p < 0.001$

age groups the serum albumin levels suggest that there was adequate protein intake which was confirmed in the food consumption survey (18). In this investigation serum albumin levels were determined as a complement to the clinical and anthropometrical investigations. However it is to be observed that serum albumin decreases late in protein deficiency states. There are still no biochemical methods for detecting early and slight degrees of malnutrition which are suitable for use in field studies on a large scale (10).

The results of the anthropometrical investigation height weight and skinfold thickness confirm that the nutritional state of the investigated children in all three age groups was good. It is interesting that the girls in the 13 year-old group had a significantly higher mean body weight than the boys. The difference was found in all three areas but it was significant only in the two rural areas (Table 6 a-d). It probably reflects the fact that girls engage in less physical activity than boys. Although the girls in all areas consumed less than the boys (18) they probably did not expend their total energy in take. In the younger age groups there were no significant sex differences in weight. Another observation is that the girls in all the age groups had significantly greater mean skinfold thickness than the boys. This is consistent with the findings in other investigations (6, 10, 11, 12, 24).

As expected weight and skinfold thickness showed significant deviation from normal distribution due to positive skewness (Figs 2 a, c and d). However tests of significance of the differences between both the means and the medians gave the same result. The coefficients of correlation (r) between triceps and subscapular skinfold thickness were 0.56 for the children in the 4 year-old group and 0.79 and 0.83 for the 8 and 13 year-old groups respectively. The coefficients of correlation (r) between triceps and subscapular skinfold thickness and weight were for the children in the 4-year-old group 0.37 and 0.32 respectively. In the 8 and 13 year-old groups on the other hand the coef-

Table 6e. *Test on the difference of the means of weight height triceps and subscapular skin folds in the different age groups and geographic areas*

1 - City of Umeå, 2 - Inland area, 3 - Mountain foreland

	Weight	Height	Triceps skinfold	Subscapular skinfold
8 year-olds				
Boys				
1 vs 2				
1 vs 3			*	
2 vs 3				
Girls				
1 vs 2			*	
1 vs 3	*	*	***	**
2 vs 3				
Total				
1 vs 2				
1 vs 3		**	***	**
2 vs 3				
13 year-olds				
Boys				
1 vs 2				
1 vs 3				*
2 vs 3				
Girls				
1 vs 2				
1 vs 3				
2 vs 3				
Total				
1 vs 2				
1 vs 3				
2 vs 3				*

* $p < 0.05$ * $p < 0.01$ * $p < 0.001$ 1 > 2 1 > 3 2 > 3

ficients were 0.66, 0.68 and 0.61, 0.66 respectively. The results for the school children are in fairly good agreement with those of Corbin on American elementary school children (4). The lower correlation in the 4 year-old group may be real or may be due to technical difficulties in measuring skinfolds in preschool children.

That the children in the city of Umeå were somewhat taller than the children in the mountain foreland may be due to nutritional differences between areas that differ socio-economically (18). There may have been geographical differences in nutritional intake in Västerbotten early in the lives of the investigated children and/or in their mothers. It cannot be estab-

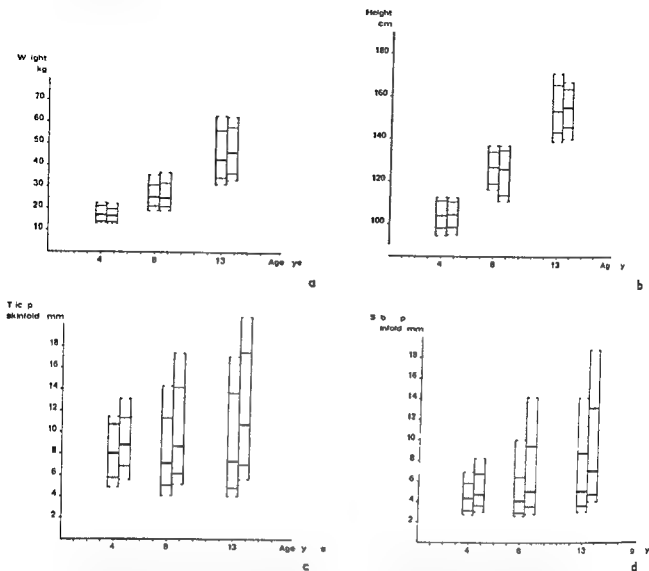


Fig 2 Percentile distribution of (a) weight (b) height (c) triceps skinfold and (d) subscapular skinfold in 4 8 and 13 year olds. The 50th percentile is indicated by a heavy line the 90th and 10th percentiles are

indicated by the solid lines above and below and the 97th and 3rd percentiles by the dotted lines at the extremes. Values for boys at the left values for girls at the right

were found between children in the different areas

Subscapular skinfold Boys in the city of Umeå had a higher mean value for the subscapular skinfold than the boys in the mountain foreland ($p < 0.05$). The children in the inland area, in turn, had a somewhat higher mean value for subscapular skinfold ($p < 0.05$) than the children in the mountain foreland (Table 6 e).

DISCUSSION

The aim of the medical examination was to determine the physical health status of the chil-

dren who took part in the food consumption survey (18), and to relate the findings to food habits and socio economic conditions (21). As expected, no case of clinical undernutrition was found. A wide variety of foods are available in the regions studied and the economic standard of the population is fairly high. It should be pointed out, however, that the methods used for evaluating health and nutritional status are rough. A clinical examination is subjective and depends on the experience and thoroughness of the investigator. However, since the same investigator examined all the children, comparisons between the areas should be valid. In all

economic conditions for growth and physical development has been demonstrated in a number of studies (e.g. 5, 6, 8, 11).

In the mountain foreland the mean values for skinfold thickness were lower than those in the city of Umeå and to some extent even than those in the inland area. Since the food consumption survey (18) showed that children in both the mountain foreland and the inland area had higher energy intakes than the children in the city of Umeå, the difference in skinfold measurement can hardly be due to a difference in nutritional intake. One is inclined to assume that this difference is mainly due to less physical activity on the part of children in Umeå. Further, a number of factors suggest that children in the city of Umeå engage in less physical activity in their daily lives. They have shorter distances to school and it is likely that their spare time activities include less physical activity whereas children in the mountain foreland probably spend more time out of doors and engage in more fresh air sports than city children. The role of inactivity in the etiology of obesity, especially in the growing child, has been pointed out by Mayer (14), Parizkova (17) and others.

Compared with earlier Swedish findings by Broman, Dahlberg & Lichtenstein (2) published in 1942 and based on sampling in 1938-1939, the mean value for body weight in the present investigation is 1-3 kg higher in all age groups. The group means for height are also 1-3 cm higher (Table 7a). For the 8-year-old group the values are in good agreement with current height and weight data from a longitudinal Swedish study by Karlberg et al. (12). In Table 7a these values are given for comparison. The 4-year-olds in Vasterbotten that is in the city of Umeå were somewhat taller than the children in Karlberg's study in Stockholm. The difference probably reflects the secular trend since the 4-year-olds in Umeå were studied 7-8 years later. For the 13-year-olds there were small differences in weight and height between the two investigations. However, the ages of the two materials for the 13-year-olds are not

exactly comparable. This must be born in mind when evaluating the differences in body measurements. It is striking that the differences between Karlberg's Stockholm children and the children in Vasterbotten are so small.

For all age groups the mean skinfold measurements were greater in the Stockholm material than in the children in Vasterbotten (Table 7b). The differences are even greater if the Stockholm children are compared only with the rural children in the present study. These differences are probably due to a number of factors such as diet, activity and socio-economic status. An analysis of the possible relation between the investigated children's general and the oral health status, food habits and socio-economic situation has been carried out and the results are described by Samuelson et al. (21).

In regard to the health status of the children there was a notably high frequency of upper respiratory infections in all age groups. Although the children were in generally good health, upper respiratory infections were present at the time of the examination or had recently been present. This was reflected in the elevated microsedimentation rates, especially among the preschool children. In the clinical examination as well it was found that the 4-year-olds had a higher frequency of symptoms of infection and enlargement of the superficial lymph nodes in the neck than did the other age groups.

The ESR values found by Mellbin (16) in Lapp children in the northern part of Sweden were of the same order of magnitude in the school children studied as in the present material. Subclinical infections were present in his material just as in the present study.

Hofvander (9) in his investigation of privileged children in Addis Ababa aged 1-14 years found a high frequency of infections which were not serious in nature but which were reflected in elevated ESR values. In Hofvander's material the mean ESR for 1 to 4-year-old children was 17.5 mm for 5 to 9-year-old children 13.3 mm and for 10 to 14-year-olds 12.7 mm. The ESR was higher than 20

Table 7a Swedish investigations on weight and height in corresponding age groups

	Sex	No	4 years 5 months				8 years 4 months				13 years 4 months			
			Weight		Height		Weight		Height		Weight		Height	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Broman	Boys		172	19	1040	43	259	35	1285	55	427	78	1536	79
Dahlberg & Lichtenstein (1942) ^a	Girls		166	20	1028	44	251	37	1270	57	443	76	1550	69
Samuelson (1971)	Boys	99	181	24	1070	47	297	26	1296	55	305	45	1570	82
	Girls	99	179	24	1065	42	286	26	1293	56	315	47	1580	67
			<i>Interpolated values</i>				<i>Interpolated values</i>				<i>Values age 13.0 years</i>			
Karlberg et al (1971)	Boys	113	178	23	1063	40	112	27	1306	54	93 ^b	43	1563	75
	Girls	83	176	22	1054	43	82	27	1292	58	64 ^b	47	1578	72

^a 3.6-4.5 years 7.6-8.5 years 12.6-13.5 years^b The children who had attained the age of 13.0 years in Nov 1970

lished, but it is not unlikely, that there were differences in the early feeding of the urban children and the children in the mountain foreland. The current nutritional situation, which was generally the same in all areas, cannot have been decisive in this respect. Although the genetic heterogeneity of the population of Vasterbotten is well documented (1) there is no conclusive evidence that the somatic differences between areas found in this study are due to genetic factors.

In the present study it has been shown that differences in socio-economic conditions play a role in the explanation of the differences in body height found between the areas (21). Mellbin (16) in his study of children of nomadic

Swedish Lapps in the northern part of Sweden, also found geographical differences in children's growth and development. The population in the northernmost area investigated had been more isolated and lived under poorer socio-economic conditions. Mellbin found that the Lapp children in the northernmost area were shorter than those farther south. It was assumed that this difference was mainly due to differences in socio-economic conditions, although genetic factors were discussed as having a certain amount of importance. Children born 1950-1953 were found to be taller for their ages than those born 1942-1946. This difference was most pronounced in the far north. In all parts of the world the importance of socio

Table 7b Swedish investigations on skinfold thickness in corresponding age groups

Table 16 Swedish investigations on skinfold thickness in developing age groups																
			4 years 5 months				8 years 4 months				13 years 4 months					
			Triceps		Subscapular		Triceps		Subscapular		Triceps		Subscapular			
	Sex	No	Mean	SD	Mean	SD	No	Mean	SD	Mean	SD	No	Mean	SD	Mean	SD
Samuelson (1971)	Boys	88	8.6	1.9	5.0	1.2	297	8.2	2.6	5.1	2.1	305	8.9	3.4	6.4	3.3
	Girls	63	9.4	1.8	5.5	1.3	286	10.1	3.3	6.5	3.5	315	12.1	4.4	8.6	4.0
			Interpolated values				Interpolated values				Values age 13.0 years					
Karlberg et al (1971)	Boys	111	9.9	1.7	5.4	1.5	112	9.2	3.3	5.0	1.8	90 ^a	10.0	4.1	7.0	4.5
	Girls	81	10.3	1.8	6.3	1.6	80	11.2	3.2	6.6	3.3	60 ^a	12.4	3.2	9.2	4.1

^a The children who had attained the age of 13.0 years in Nov 1970

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mm in 28.1% of the 1 to 4-year-olds, in 15.4% of the 5 to 9 year olds and in 12.3% of the 10 to 14-year olds. Both the mean values and the frequencies of ESR values above 20 mm are in fairly good agreement with the findings in the present study.

For the 8-year-olds, the difference in mean ESR's between the inland area and the mountain foreland was very small. It was probably due to a somewhat higher incidence of upper respiratory infections in the mountain foreland during the 4 month period of investigation.

The other findings in the clinical investigation were less remarkable. The frequency of eczema for instance in this study was found to be 6.1%. In a health screening program of 4 year olds resident in another county of Sweden Vuille (25) found about the same frequency (6.0%). All the children were receiving preventive health care through child health centres and school health services.

In the haematological investigation of the children (20), the incidence of iron deficiency anaemia was found to be very low in all age groups. Only two 13-year-old girls were found to have depleted iron stores, mainly because of heavy losses of iron in menstruation.

In summary, the health and nutritional status of both the rural and urban children were found to be good. This no doubt reflects the availability of an abundance of foods of various kinds in the areas studied, the increasingly comprehensive nature of preventive health services, and the successive improvement in socio-economic conditions.

SUMMARY

The health and nutritional status of 1 401 children aged 4, 8 and 13 years in one urban area and two rural areas of a northern Swedish county were studied.

In none of the age groups was there any undernutrition. Anthropometrical measurements reflected the good nutritional status, with height and weight generally conforming to current values of a Swedish urban population. The girls

had significantly thicker skinfolds than the boys. The children in the city of Umeå had thicker skinfolds than the children in the mountain foreland and were also significantly taller.

There was a rather high frequency of subclinical upper respiratory infections among the children, especially among the 4 year olds.

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Table 1 Results of the screening study among newborn babies

Total no of children tested	Blood phenylalanine		
	<4 mg/ 100 ml	4-6 mg/ 100 ml	>6 mg/ 100 ml
71 135	71 131	24	0

90% of which were idiots or imbeciles) have thus far been screened with the bacterial inhibition test. With two cases of PKU among them a prevalence of 0.54 per 1 000 is obtained. The average incidence of PKU among institutionalized mentally retarded patients in 12 countries was 0.64 / (6) and the results from the Scandinavian countries fall very close to that figure. The difference between these prevalences is significant at the level $p < 0.001$ as tested with the Poisson's distribution. That the incidence of PKU among the Finnish newborn does not exceed one per 100 000 is well established. The Finns thus fall into the same category as the American blacks (1).

Finland with her total population of 4 800 000 has a rather remote location in Northern Europe. The immigration rate is very low and the main population stock comes from the East Baltic and Nordic races. The ethnic background thus differs from that of the other Scandinavian countries. The closest ethnic relatives the Fenno-Ugrians, mostly live in the Soviet Union from where no published data on PKU are available. No cases of PKU have been found in Estonia (3) but a screening study among the Hungarians who also have ethnic ties with the Finns revealed three cases of PKU among 20 000 newborns (7). The Finns obviously have a very special genetic background. Many rare diseases such as congenital nephrosis, selective vitamin B₁₂ malabsorption, congenital chloridorrhoes, protein intolerance with lysinuria and aspartylglucosaminuria have been found more frequently in Finland than in the rest of the world. On the other hand some other diseases such as cystic fibrosis, galac-

tosaemia and PKU are much less common than in other European countries.

Some error may have been introduced into the present screening system because a number of premature and sick infants escaped the sampling. However it is not known that the birth weight of PKU patients is lower than that of other infants nor is there any reason to expect them to have more difficulty during the neonatal period than normal babies. As advocated by Hsia & Dobson (8) in all cases the Guthrie test was performed later than 48 hours and only after 24 hours of feeding with proteins. Therefore the results obtained were not due to lack of feeding.

The screening of newborn babies was stopped during May 1970 because of the extremely low incidence of the disease.

SUMMARY

Guthrie's bacterial inhibition test was applied to 71 135 newborn infants from different parts of Finland. No cases of PKU were diagnosed. In addition 3 685 mentally retarded patients, mostly idiots and imbeciles, have been screened in Finland with the same method. With two cases of PKU among them a prevalence of 0.54 per 1 000 is obtained. The incidence of the disease among the newborn is estimated to be less than one per 100 000.

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THE INCIDENCE OF PKU IN FINLAND

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The incidence of phenylketonuria (PKU) among newborn babies is approximately one per 10 000 in most Western countries. It is racially determined, and some very low figures have also been reported, e.g. among the American blacks (1) and among the Ashkenazi Jews (2). The incidence was assumed to be of the same order in Finland as in the Scandinavian countries. However, only three cases had been diagnosed through all time by 1966 and this led to the suggestion that PKU might be an exceptionally rare disease in Finland (3). To estimate the true incidence of this disease, wide range screening of the newborn was initiated. In addition, a number of institutionalized patients were studied. This paper reports the results of these studies.

MATERIAL AND METHODS

Twelve obstetric hospitals in various parts of the country took part in the project. After careful instruction of the nurses and laboratory technicians, the first blood specimens taken by heel puncture were sent to the central laboratory on Nov 11 1966. By April 30 1970 a total of 71 135 newborn infants had been tested, representing about one third of the babies born during the same period in Finland. Glucose solution was given to all babies 4 to 24 (average 12) hours and breast milk 24 hours after birth. Mothers' milk was also given when breast feeding was not possible, except in a few instances when cow's milk was given. The blood sample was taken a minimum of 96 hours after the birth. Premature or sick children who had been transferred to other wards or hospitals were excluded from the series. In addition to the newborn babies, a total of 1 595 patients at various institutions for the

mentally retarded were also screened. The patients were taken at random but approx 90% were profoundly severely or moderately retarded (idiots and imbeciles). Serum phenylalanine was determined by the conventional bacterial inhibition test (4). Samples with a known elevated phenylalanine level were occasionally sent to the laboratory as routine specimens for control purposes. The reliability of the method was verified with a fluorimetric procedure (5).

RESULTS

The results of the screening programme among the 71 135 newborn babies are presented in Table 1. No case of permanent hyperphenylalaninaemia was discovered. Blood phenylalanine levels of between 4 mg/100 ml and 6 mg/100 ml were observed in 24 babies, but the result was normal in every instance when a control sample was tested.

No cases of PKU were found among the 1 595 institutionalized mentally retarded patients.

DISCUSSION

Because no cases of PKU were detected among the newborn, the figures obtained from the screening of the mentally retarded patients must be utilized in an estimation of the prevalence of the disease in Finland. A total of 2 090 patients were screened earlier, among whom two cases of PKU were detected (3) and in addition 1 595 cases were examined now, so that a total number of 3 685 institutionalized mentally retarded patients (approx

RENAL FUNCTION TESTS IN NONACUTE RECURRENT URINARY TRACT INFECTIONS IN CHILDREN

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Children with recurrent urinary tract infections examination of renal function is of utmost importance in following the natural progression of the disease. Clearance studies are presently considered to be the most valid techniques for determining renal function. This type of studies must be restricted for several reasons. Most patients with recurrent urinary tract infections will therefore be followed by both blood and urine analyses which will only give qualitative estimates of either glomerular or tubular function. In the present study the results from various clearance studies have been compared with the results of blood and urine analyses. The correlation between glomerular and tubular function has also been studied.

MATERIAL

15 patients, 4 boys and 11 girls, aged 3 1/2 to 17 1/2 years have been studied. All the patients had histories of recurrent urinary tract infections confirmed by urine cultures. They were all without any clinical or radiological signs of infection the last two months prior to the presented studies. The results from the clearance studies have been given in previous reports (1, 2, 3, 5, 6). The blood and urine tests were obtained at the same period of time (within 2 weeks) as the clearance studies.

METHODS

Glomerular filtration rate

Blood tests For the analysis of serum creatinine and blood urea nitrogen blood samples were withdrawn

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in the morning with the patients fasting. Serum creatinine was analysed in venous blood samples. 5 ml blood was required. Blood urea nitrogen was analysed in capillary blood samples. 0.1 ml blood was required.

Clearance studies Glomerular filtration rate was determined by the clearance of inulin (14). For this purpose a continuous infusion of 10% inulin (Laeva-sar-Gesellschaft) 0.001 g/min/kg body weight was given following the prime dose of 0.05 g/kg b.w. The continuous infusion was begun 60 min before the first urine collection period was started in order to obtain saturation of extracellular volume with inulin. For urine sampling a double lumen polyethylene catheter was used enabling a continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein.

Concentrating capacity

Fluid deprivation tests One to three urine samples obtained by spontaneous voiding were taken after the patients had been deprived of fluid and food for 19 hours. In some of the patients the fluid deprivation test was repeated but at this time with the intramuscular injection of Pitressin tannate® (Parke & Davis) in a dosage of 0.5 pressor units per 6 kg body weight (16). The urine specimens were analysed for osmolality. The maximal urine osmolality was used for further analysis.

Clearance studies Following 19 hours of water deprivation 2-3 urine collection periods were obtained each of about 45 min. Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder. Venous blood samples were withdrawn in the middle of each urine collection period. The studies were performed during the continuous infusion of inulin enabling simultaneous determination of filtration rate. Urine and blood samples were analysed for osmolality and inulin.

Diluting capacity

Induction of water diuresis The aim of this procedure was to eliminate the effect of endogenous

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In children with recurrent urinary tract infections examination of renal function is of utmost importance in following the natural progression of the disease. Clearance studies are presently considered to be the most valid techniques for determining renal function. This type of studies must be restricted for several reasons. Most patients with recurrent urinary tract infections will therefore be followed by both blood and urine analyses which will only give qualitative estimates of either glomerular or tubular function. In the present study the results from various clearance studies have been compared with the results of blood and urine analyses. The correlation between glomerular and tubular function has also been studied.

MATERIAL

33 patients, 4 boys and 29 girls aged 3 $\frac{1}{2}$ to 17 $\frac{1}{2}$ years have been studied. All the patients had histories of recurrent urinary tract infections confirmed by urine cultures. They were all without any clinical or bacteriological signs of infection the last two months prior to the presented studies. The results from the clearance studies have been given in previous reports (2, 3, 5, 6). The blood and urine tests were obtained at the same period of time (within 2 weeks) as the clearance studies.

METHODS

Glomerular filtration rate

Blood tests: For the analysis of serum creatinine and blood urea nitrogen blood samples were withdrawn

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in the morning, with the patients fasting. Serum creatinine was analysed in venous blood samples. 5 ml blood was required. Blood urea nitrogen was analysed in capillary blood samples. 0.1 ml blood was required.

Clearance studies: Glomerular filtration rate was determined by the clearance of inulin (14). For this purpose a continuous infusion of 10 inulin (Laeva sar Gesellschaft) 0.001 g/min/kg body weight was given following the prime dose of 0.05 g/kg b.w. The continuous infusion was begun 60 min before the first urine collection period was started in order to obtain saturation of extracellular volume with inulin. For urine sampling a double lumen polyethylene catheter was used enabling a continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein.

Concentrating capacity

Fluid deprivation tests: One to three urine samples obtained by spontaneous voiding were taken after the patients had been deprived of fluid and food for 19 hours. In some of the patients the fluid deprivation test was repeated but at this time with the intramuscular injection of Pitressin tannate® (Parke & Davis) in a dosage of 0.5 pressor units per 6 kg body weight (16). The urine specimens were analysed for osmolality. The maternal urine osmolality was used for further analysis.

Clearance studies: Following 19 hours of water deprivation 2-3 urine collection periods were obtained each of about 45 min. Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder. Venous blood samples were withdrawn in the middle of each urine collection period. The studies were performed during the continuous infusion of inulin enabling simultaneous determination of filtration rate. Urine and blood samples were analysed for osmolality and inulin.

Diluting capacity

Induction of water diuresis: The aim of this procedure was to eliminate the effect of endogenous

antidiuretic hormone (12) For this purpose the patients were asked to drink water in an amount corresponding to 2-2.5% of body weight in an hour and thereafter in amounts corresponding to 0.5 of body weight every 30 min When the diuresis was constant which generally occurred 90 min after the intake of fluid was started urine collection was begun

Test of minimum urine osmolality Following the induction of water diuresis i.e. when the diuresis was constant 2-3 spontaneously voided urine samples were obtained The specimens were analysed for osmolality The means of the determinations obtained were used for further analysis

Clearance studies Following the induction of water diuresis i.e. when the diuresis was constant 3-4 urine collection periods each of about 15 min were obtained Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period The studies were performed during the continuous infusion of inulin Thus simultaneous determinations of the filtration rate could be obtained

Sodium homeostasis

Blood tests Venous blood samples were withdrawn in the morning while the patients were fasting The blood samples were analysed for sodium

Urine tests The urine samples were those obtained during the fluid deprivation tests The urine specimens were analysed for sodium

Clearance studies Clearance studies were performed during hydropenia i.e. after 19 hours of water deprivation The studies were performed during the continuous infusion of inulin thus allowing simultaneous determinations of filtration rate Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period

Renal regulation of acid base balance

Blood tests Prewarmed capillary blood samples were taken in the morning for the analysis of standard bicarbonate

Short time ammonium chloride test (8) The study was started after a standard breakfast meal In order to keep a constant urine flow rate the patients were given water in an amount of 0.1-0.2 ml/min/kg body weight The forced fluid intake was started 2-4 hours before the intake of ammonium chloride Urine was collected hourly by spontaneous voiding Following control sampling of blood and urine ammonium chloride was given in the amount of 150 mEq/m² body surface After the administration of the ammonium chloride another five urine samples were collected and two to three blood samples were taken 2, 3 and 5 hours after the administration Immediately after voiding the urine samples were withdrawn and kept anaerobically on ice pH analysis

were carried out within 15 min after voiding Prewarmed capillary blood samples were taken and analysed for actual pH in samples equilibrated with 4 and 8 CO₂ By plotting those data on a Siggaard Andersen curve nomogram Pco₂, standard bicarbonate and total CO₂ could be obtained (13)

Analytical methods

Serum creatinine was determined as true creatinine with the Jaffe reaction after adsorption on to Lloyds reagent Blood urea nitrogen was determined by analysing for ammonia colorimetrically before and after the action of urease (9) Inulin in blood and urine was determined according to the method of Heyrovsky (11) Osmolality in blood and urine was determined cryoscopically with a Knauer osmometer Sodium in blood and urine was determined with a flame photometer (Eppendorf) pH in urine was determined on a pH meter 26 (Radiometer) pH in blood was determined on a pH meter 27 (Radiometer)

Calculations

The clearance of inulin was calculated from the formula

$$C_{In} = \frac{U_{In} \times V}{B_{In}}$$

where U_{In} and B_{In} stand for the concentrations of inulin in urine and blood respectively V stands for the diuresis

Free water reabsorption (T_{H_2O}) was calculated according to the formula

$$T_{H_2O} = C_{osm} - V$$

where C_{osm} is calculated from the formula

$$C_{osm} = \frac{U_{osm} \times V}{B_{osm}}$$

where U_{osm} and B_{osm} stand for the osmolality in urine and blood respectively V see above

Free water clearance (C_{H_2O}) is calculated from the formula

$$C_{H_2O} = V - C_{osm}$$

Symbols see above

Fractional sodium excretion (C_{Na}/C_{In}) is calculated from the formula

$$C_{Na}/C_{In} = \frac{U_{Na} \times V \times 100}{B_{Na} \times C_{In}}$$

where U_{Na} and B_{Na} stand for the concentrations of sodium in urine and blood respectively V and C_{In} see above

Total CO₂ concentration = actual bicarbonate concentration + 0.0306 × actual Pco₂ Student's t test has been used for the statistical analysis

RESULTS AND COMMENTS

Glomerular filtration rate

Two blood tests are used for screening of glomerular filtration rate, namely serum creatinine concentration and blood urea nitrogen

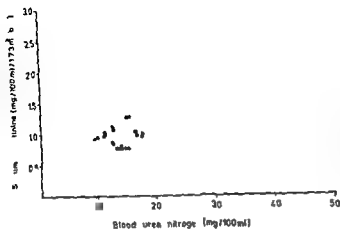


Fig 1 Relationship between blood urea nitrogen concentration (mg/100 ml) and serum creatinine concentration (mg/100 ml) 173 m b.s. 41 observations in 32 patients are included

concentration. The advantage with the determination of serum creatinine is its relative stability despite variations in the protein intake (4). The analytical method is however time consuming and generally venous blood samples are needed for the analysis. The advantage with blood urea nitrogen determination is the simple method of analysis and the small amounts of blood required. The disadvantages with blood urea nitrogen determination are however the variations of blood urea nitrogen concentration with protein intake and water content of the body (1, 15). Fig 1 demonstrates the relationship between blood urea nitrogen and serum creatinine concentration. Since serum creatinine is related in a linear

fashion to body surface (7) the values have been corrected for 1.73 m b.s. When serum creatinine is below 13 mg/100 ml which is considered to be the upper limit of normal and blood urea nitrogen concentration is below 20 mg/100 ml there is no apparent relationship between serum creatinine and blood urea nitrogen. In fact variations between serum creatinine concentration and blood urea nitrogen concentration are even found in each individual when followed continuously as demonstrated in Fig 2. When blood urea nitrogen concentration exceeds what is considered to be upper limit of normal (20 mg/100 ml) the correlation between blood urea nitrogen concentration and serum creatinine concentration is fairly good

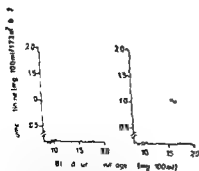


Fig 2 Relationship between blood urea nitrogen concentration (mg/100 ml) and serum creatinine concentration (mg/100 ml) 173 m b.s. in two patients on 7 and 6 different occasions respectively

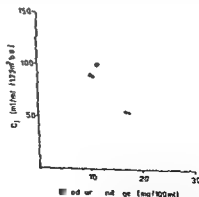


Fig 3 Relationship between blood urea nitrogen concentration (mg/100 ml) and clearance of inulin (C_{in} ml/min/1.73 m b.s.) during hydropenia 27 observations in 24 patients are included

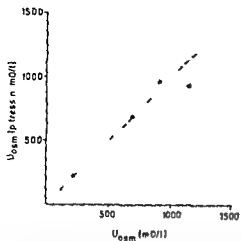


Fig 4 Relationship between urine osmolality (U_{osm} mO/l) after 19 hours of water deprivation and urine osmolality after pitressin tannate was injected followed by 19 hours of water deprivation. 11 patients are included. The dashed line indicates the isosmotic line.

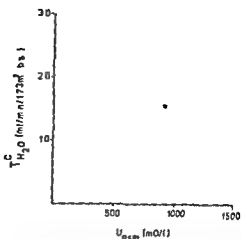


Fig 5 Relationship between urine osmolality (U_{osm} mO/l) after 19 hours of water deprivation and free water reabsorption (T_{H_2O} ml/min/1.73 m² bs). 25 observations in 21 patients are included.

(correlation coefficient 0.97, highly significant). Thus no difference between the two methods can be noticed. Presently therefore blood urea nitrogen determination can be recommended in pediatric praxis because the small amount of blood required for the analysis. Fig 3 demonstrates the relationship between blood urea nitrogen concentration and the clearance of inulin during hydropenia. The state of hydropenia was chosen because the blood test was taken in the morning with the patients fasting. An increase in blood urea nitrogen concentra-

tion is generally not noted until the glomerular filtration rate is reduced below 50 ml/min/1.73 m² bs. This is in accordance with the results of de Wardener (15) who studied the relationship between clearance of creatinine and blood urea nitrogen in adults. A normal value of blood urea nitrogen will thus only mean that the filtration rate is above 50 ml/min/1.73 m² bs.

Concentrating capacity

Fig 4 demonstrates the relationship between urine osmolality after 19 hours of fluid deprivation and urine osmolality after pitressin tannate was given in combination with fluid deprivation. The time interval between the two tests does not exceed a week. The scatter of the results is fairly large but there is no typical trend in the deviations. Fig 5 demonstrates the relationship between urine osmolality after 19 hours of fluid deprivation and free water reabsorption. There is a highly significant positive relationship between free water reabsorption and maximal urine osmolality; correlation coefficient 0.63. It should be noted that maximal urine osmolality and free water reabsorption are measurements of two related but not identical determinants of concentrating capacity. The maximal urine osmolality is a measurement of the tonicity of the renal medulla

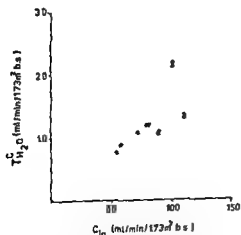


Fig 6 Relationship between glomerular filtration rate (C_I ml/min/1.73 m² bs) during hydropenia and free water reabsorption (T_{H_2O} ml/min/1.73 m² bs). 25 observations in 21 patients are included.

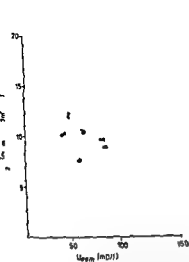


Fig 7 Relationship between minimum urine osmolality (U_{0sm} mOsm/l) and free water clearance (C_{H_2O} ml/min/1.73 m² b s) 37 observations in 28 patients are included

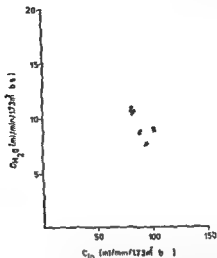


Fig 8 Relationship between glomerular filtration rate (C_{in} ml/min/1.73 m² b s) during water diuresis and free water clearance (C_{H_2O} ml/min/1.73 m² b s) 37 observations in 28 patients are included

and thus an index of the function of the loop of Henle. Determination of free water reabsorption is a method of quantitating the principal aim of urine concentration i.e. fluid conservation. In hydropenia the kidneys excrete a urine which is more concentrated than the blood. The glomerular filtrate has the same osmotic concentration as the plasma (10). In order to concentrate the urine the renal tubules must remove a certain quantity of solute free water from the isosmotic glomerular filtrate. This volume of water removed can be calculated by the difference between the volume of urine required to contain urinary solutes in an isosmotic state i.e. the osmolar clearance C_{osm} and the urine flow V . The free water reabsorption can be defined as the volume of water reabsorbed in excess of an isosmotic equivalent of solute. Free water reabsorption is thus a qualitative and a quantitative measurement of concentrating capacity. By determining free water reabsorption the reserve capacity of the loop of Henle can be estimated in the limits of increased solute loading. The reserve capacity cannot be assessed by the determination of urine osmolality only. For clinical purpose however, determination of maximal urine

osmolality is sufficient for the evaluation of the concentrating capacity provided there is no osmotic diuresis as in glucosuria. Fig. 6 demonstrates the relationship between glomerular filtration rate determined by the clearance of inulin during hydropenia and free water reabsorption. When filtration rate is reduced a depressed free water reabsorption is noticed. The correlation coefficient is 0.75 which is highly significant. Thus there is no great discrepancy between filtration rate and the tubular function dealing with concentration of urine in recurrent urinary tract infections.

Diluting capacity

Fig 7 demonstrates the relationship between minimum urine osmolality and free water clearance. With decreasing urine osmolality an increase in free water clearance is seen. The correlation coefficient is 0.58 which is highly significant. Minimum urine osmolality and free water clearance are measurements of two related but not identical determinants of diluting capacity. The minimum urine osmolality is a qualitative measurement of diluting capacity and thus an index of the function of the ascending limb of Henle and the first part of distal

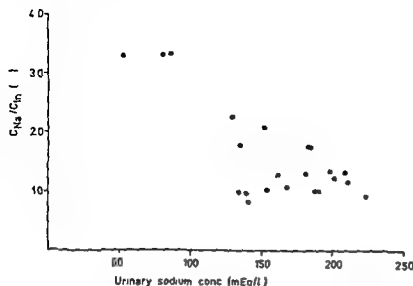


Fig 9 Relationship between urinary sodium concentration (U mEq/l) and fractional sodium excretion (C_{Na}/C_m %) during hydropenia. 24 observations in 20 patients are included.

convoluted tubule. Free water clearance measures the amount of water being discharged from solutes when passing through the nephron mainly the ascending limb of Henle and the early distal tubule. Thus free water clearance is both a qualitative and a quantitative measurement of the function of these parts of the nephron. In contrast to minimum urine osmolality free water clearance can therefore be used for evaluating the reserve capacity in the ascending limb of Henle and early distal tubule. For clinical purpose however urine osmolality is sufficient for evaluating the diluting capacity. Fig 8 demonstrates the relationship between glomerular filtration rate determined by the clearance of inulin during water diuresis and free water clearance. There is a highly significant positive relationship between free water clearance and glomerular filtration rate correlation coefficient 0.65. This indicates that there is a rather homogenous reduction of the glomerular and distal tubular functions in recurrent urinary tract infections in children.

Sodium homeostasis

Serum sodium concentration was within normal limits, 133–146 mEq/l, in all patients studied. Fractional sodium excretion, however, which is normally below 1.8% (2) was in the range of 2.1 to 3.5% in 5 patients. This increase in fractional sodium excretion did apparently not

result in hyponatremia. Fig 9 demonstrates the relationship between urine sodium concentration and fractional sodium excretion. The values were obtained during hydropenia. With increasing fractional sodium excretion there is a decrease in urinary sodium concentration. Thus in contrast to what has generally been considered determination of urine sodium concentration might be a valuable tool in the assessment of renal function. This assumes that urinary sodium concentration is determined during a controlled diuretic state e.g. maximal hydropenia. It is therefore suggested that urine sodium concentration should be routinely examined during a conventional fluid deprivation test. The mean urine sodium concentration during hydropenia in patients with filtration rates above 80 ml/min/1.73 m² b.s. and fractional sodium excretion below 1.8% is 179 ± 56 mEq/l (mean value ± 2 SD). A deviation in urine sodium concentration below -2 SD, i.e. 123 mEq/l then strongly suggests increased fractional sodium excretion. Fig 10 demonstrates the relationship between glomerular filtration rate and fractional sodium excretion during hydropenia. With decreasing filtration rates there is an increase in fractional sodium excretion. This might be a sign of a homogeneous reduction of glomerular and tubular functions but reports from this laboratory have indicated that the relationship between

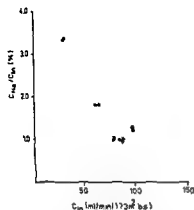


Fig 10 Relationship between glomerular filtration rate (C_m ml/min/1.73 m² bs) and fractional sodium excretion (C_{Na}/C_i) during hydropema. 24 observations in 10 patients are included

filtered load of sodium and reabsorbed sodium might rather be a physiologically induced reset of glomerular tubular balance for sodium (3)

Renal regulation of acid base balance

The contribution of the kidneys to the maintenance of the acid base balance is accomplished by the secretion of hydrogen ions. These hydrogen ions either participate in the reabsorption of bicarbonate or are excreted in the form of ammonium ions or titratable acids. Fig 11 demonstrates the relationship between basal standard bicarbonate concentration and

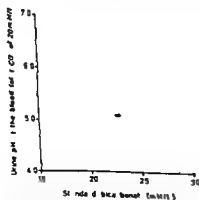


Fig 11 Relationship between the basal standard bicarbonate concentration (mM/l) and the estimated urine pH at the blood total CO₂ concentration of 0 mM/l. 16 patients are included

the urine pH value when the blood total CO₂ concentration by an oral acid load has been reduced to 20 mM/l. In healthy children the urine pH at this blood total CO₂ concentration of 20 mM/l is below 5.5. No good relationship can be found between the urine pH at the total CO₂ concentration of 20 mM/l and the standard bicarbonate concentration in children with recurrent urinary tract infections. Thus 7 of 12 patients with normal standard bicarbonate levels had signs of some defect in urinary acidifying mechanisms, i.e. urine pH value above 5.5 at the total CO₂ concentration of 20 mM/l. Previously we have shown that a defect in the reabsorption of filtered bicarbonate is most likely the most prominent change in urinary acidifying mechanisms (6). Since the majority of patients do not show any signs of acidosis it is probable that the degree of the defect in bicarbonate reabsorption varies from nephron to nephron and that some of the nephrons are still capable of a practically normal function in respect to bicarbonate reabsorption. This would explain why the urinary bicarbonate loss in most patients is sufficiently small not to result in renal acidosis.

Fig 12 demonstrates the relationship between glomerular filtration rate during water diuresis and the urine pH at a total CO₂ concentration of 20 mM/l. It is highly noticeable

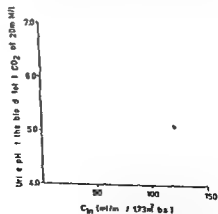


Fig 12 Relationship between glomerular filtration rate (C_m ml/min/1.73 m² bs) during water diuresis and the estimated urine pH at the total CO₂ concentration of 20 mM/l. 16 patients are included

that 5 of 9 patients with normal filtration rates demonstrate a defect in urinary acidifying mechanisms

Thus determination of renal acidifying capacity might be the most sensitive tool in the assessment of renal function in recurrent urinary tract infections in children. Again it should be postulated that no quantitative estimation of renal hydrogen ion secretion could be done with the short time ammonium chloride test.

CONCLUSION

Previous studies from this laboratory have shown that changes in renal function in children with recurrent urinary tract infections practically always occur when renal parenchymal changes can be demonstrated with an intra venous pyelogram. On the basis of the present results it is recommended that these patients at least yearly are followed by the following tests: blood urea concentration, fluid deprivation test with determination of urine osmolality and of urine sodium concentration, water load test with determination of urine osmolality and a short time ammonium chloride test. If possible a clearance study with the determination of the glomerular filtration rate should also be carried out.

SUMMARY

The clinical value of various renal function tests in recurrent urinary tract infections in children has been examined. The study also includes an evaluation of glomerular capacity versus tubular capacity in recurrent urinary tract infections. There was no good correlation between blood urea nitrogen concentration and serum creatinine concentration within the normal limits for these two parameters. When blood urea nitrogen concentration and/or serum creatinine concentration were elevated a highly significant correlation between the two parameters was found. When relating blood urea nitrogen concentration to the clearance of inulin an elevation of blood urea nitrogen con-

centration was not found until the filtration rate was below 50 ml/min/1.73 m² b.s. The tubular functions were examined by studies of (a) the concentrating capacity, (b) the diluting capacity, (c) sodium reabsorption and (d) renal acid base regulation. The concentrating capacity was determined by two screening tests, maximal urine osmolality after 19 hours of fluid and food deprivation with or without injection of pitressin tannate, and by free water reabsorption. The administration of exogenous pitressin had no significant effect on maximal urine osmolality. There was a highly significant correlation between free water reabsorption and maximal urine osmolality. A highly significant correlation was also found between free water reabsorption and glomerular filtration rate. Diluting capacity was evaluated by determining minimum urine osmolality and free water clearance. A highly significant correlation between minimum urine osmolality and free water clearance was obtained. There was a good correlation between free water clearance and glomerular filtration rate. When sodium reabsorption is depressed below normal, urine sodium concentration during hyponatremia is abnormally low. A normal standard bicarbonate level does not exclude a defect in renal acidifying mechanisms. A defect renal acidifying capacity could be found even in patients with normal filtration rates indicating that renal acidifying capacity might be the first sign of renal damage in children with recurrent urinary tract infections.

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Table 1 Plasma growth hormone and growth response to human growth hormone

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Pat	Sex	Age (y)	Max GH level during ITT $\mu\text{g/ml}$	Max GH increase during ITT $\mu\text{g/ml}$	Growth velocity 6 months prior to therapy cm/yr	Growth velocity first 6 months on therapy cm/yr
<i>Primordial growth retardation</i>						
Sch D	♂	7	13.5	+ 6.9	5.4	7.3
M Ch	♂	7½	29.0	+26.0	4.8	6.1
A A	♂	10	32.0	+32.0	4.1	10.0 ^a
<i>Hypopituitary growth retardation</i>						
C M	♂	7	1.6	+ 1.1	3.4	10.2
W D	♂	11	0.9	+ 0.3	2.7	10.6 ^b
Co M	♂	15	0.8	0	2.4	13.1
Co P	♂	16	0.7	- 0.1	3.5	10.4

ITT Insulin tolerance test intravenously 4 Units Actrapid Novo,m²

Growth spurt due to spontaneous onset of puberty

^a Patient receiving thyroid in addition to growth hormone because of documented TSH deficiency

ministration of 0.33 g glucose/kg bodyweight as a 40% solution over a one minute period (14)

On a separate day a one hour glucose infusion test (modified method of Cerasi & Luft (4)) was performed as follows. It was started with a priming dose calculated to reach an initial glucose level of 260 mg/100 ml. Immediately after the priming dose a glucose infusion was administered by a syringe type constant pump (Braun Melsungen) at a flow rate of 120 ml/hour and at a concentration calculated from the K value in order to obtain a constant glucose level at approximately 260 mg/100 ml during a 60 min period (mean dosage 21.2 mg/kg/min range 15.8-28.8). After 60 min the glucose infusion was replaced by a normal saline infusion at the same rate. Blood

was drawn at -15 0 +15 +30 +45 +60 +90 +120 +150 and -180 min through an indwelling plastic or siliconized needle and was immediately chilled in ice water. Glucose was determined by a glucoseoxidase method (21) free fatty acids by a titrimetric method (24) growth hormone by a double antibody radioimmunological method (28) from plasma. Insulin was assessed by a double antibody radioimmunological method (11, 17) from serum.

During one week prior to the examination the usual weekly dosage was divided into daily administrations (e.g. 1.43 mg/m²/injection) and on the day of the test the dose was given exactly 60 min prior to the beginning of the glucose infusion.

The results were plotted on millimeter paper the

Table 2 Influence of growth hormone on glucose disappearance rate (K value)

	Prior to therapy	6 months	12 months	18 months	27 months
<i>Primordial growth retardation</i>					
Sch D	2.66	1.82			
M Ch	2.16	3.64			
A A	2.16	3.04			
<i>Hypopituitary growth retardation</i>					
C M	2.43	2.48	2.57	3.14	2.56
W D	3.4	2.31	2.40	2.48	2.16
Co M			1.54	1.54	1.78 ^a
Co P			2.10	1.87	

No malchliben

n=70 age 3-14 yrs 2.04±0.31 (SD)

On 1- mg HGH day

INFLUENCE OF LONG TERM GROWTH HORMONE THERAPY ON GLUCOSE TOLERANCE AND INSULIN SECRETION

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Growth rate in hypopituitary growth retardation is successfully accelerated by the administration of human growth hormone (HGH) (19, 20, 23, 25). Since this treatment has to be continued for a prolonged period of time, it is important to take into consideration all its possible side effects.

The diabetogenic effect of growth hormone (GH) was first demonstrated by Young in 1937 (26): dogs receiving injections of extracts of anterior pituitary glands developed permanent so-called metahypophyseal diabetes. Endogenous overproduction of GH in acromegaly is frequently associated with a diabetic condition (6, 12) which is characterized by a decreased glucose tolerance in the presence of hyperinsulinism (1, 5, 7, 8). Exogenous HGH in a dosage which is probably well above the physiological range was shown to produce similar derangements of carbohydrate metabolism in healthy humans (16) as well as in panhypopituitarism (15).

The dosage of HGH commonly used in the treatment of hypopituitary growth retardation is assumed to be physiological on a purely empirical basis. Furthermore, for practical reasons the weekly HGH dosage is usually given in two or three injections only. Such a schedule necessarily leads to markedly elevated plasma levels, which probably remain pathologically elevated for several hours after intramuscular

administration (18). A longitudinal study of carbohydrate metabolism during HGH substitution therapy is therefore of interest.

In the present investigation the effect of chronic administration of HGH (Raben) on glucose mediated insulin release and plasma free fatty acid concentration was studied repeatedly in three children with primordial growth retardation and in four children with documented GH deficiency.

PATIENTS

The diagnosis in the three patients with primordial growth retardation (M. Ch., Sch. D., A. A.) and the four with hypopituitary growth retardation (C. M., W. D., Co. M., Co. P.) was based on radioimmunological determination of GH in plasma during insulin tolerance tests. The maximal rise of GH is given in Table 1. Further details and the clinical findings are reported in a separate communication (13). The investigation was carried out repeatedly over periods of 6 to 27 months after onset of HGH therapy (Raben batches 22, 23) which was administered intramuscularly at a dosage of 10 mg/m²/week in three injections: 3×3.33 mg/m²/week unless otherwise stated. Growth response was slight in the children with primordial and good in those with hypopituitary growth retardation (Table 1).

For comparison a 52 year old male patient (K. E.) with acromegaly was studied prior to and three months after surgical removal of a pituitary adenoma¹.

METHODS

The K value for the glucose disappearance rate was determined after overnight fasting by intravenous ad-

¹ This patient was kindly referred to us by PD Dr M. P. König, Department of internal medicine University of Bern.

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Table 1 Plasma growth hormone and growth response to human growth hormone

Pat	Sex	Age (y)	Max GH level during ITT mg/ml	Max GH increase during ITT mpµg/ml	Growth response to human growth hormone	
					Growth velocity 6 months prior to therapy cm/yr	Growth velocity first 6 months on therapy cm/yr
<i>Primordial growth retardation</i>						
Sch D	♂	7	13.5	+ 6.9	5.4	7.3
M Ch	♂	7½	29.0	+26.0	4.8	6.1
A A	♂	10	32.0	+32.0	4.1	10.0 ^a
<i>Hypopituitary growth retardation</i>						
C M	♂	7	1.6	+ 1.1	3.4	10.2
W D	♂	11	0.9	+ 0.3	2.7	10.6 ^b
Co M	♂	15	0.8	0	2.4	13.1
Co P	♂	16	0.7	- 0.1	3.5	10.4

ITT Insulin tolerance test intravenously 4 Units Actrapid Novo/m²^a Growth spurt due to spontaneous onset of puberty^b Patient receiving thyroid in addition to growth hormone because of documented TSH deficiency

ministration of 0.33 g glucose/kg bodyweight as a 40% solution over a one minute period (14).

On a separate day a one hour glucose infusion test (modified method of Cerasi & Luft (4)) was performed as follows. It was started with a priming dose calculated to reach an initial glucose level of 260 mg/100 ml. Immediately after the priming dose a glucose infusion was administered by a syringe type constant pump (Braun Melsungen) at a flow rate of 1.0 ml/hour and at a concentration calculated from the K value in order to obtain a constant glucose level at approximately 260 mg/100 ml during a 60 min period (mean dosage 21.7 mg/kg/min range 15.8-28.8). After 60 min the glucose infusion was replaced by a normal saline infusion at the same rate. Blood

was drawn at 15.0 +15 +30 +45 +60 +90 +120 +150 and +180 min through an indwelling plastic or siliconized needle and was immediately chilled in ice water. Glucose was determined by a glucoseoxidase method (21), free fatty acids by a titrimetric method (24), growth hormone by a double antibody radioimmunological method (28) from plasma. Insulin was assessed by a double antibody radioimmunological method (11, 17) from serum.

During one week prior to the examination the usual weekly dosage was divided into daily administrations (e.g. 1.43 mg/m²/injection) and on the day of the test the dose was given exactly 60 min prior to the beginning of the glucose infusion.

The results were plotted on millimeter paper the

Table 2 Influence of growth hormone on glucose disappearance rate (K value)

	Prior to therapy	6 months	12 months	18 months	27 months
<i>Primordial growth retardation</i>					
Sch D	2.66	1.82			
M Ch	2.16	3.64			
A A	2.16	3.04			
<i>Hypopituitary growth retardation</i>					
C M	2.48	2.48	2.57	3.14	2.56
W D	3.47	2.31	2.40	2.43	2.16
Co M			1.54	1.54	1.78*
Co P			2.10	1.87	
<i>Normal children</i>					
n = 20 age 3-12 yrs. 2.04 ± 0.51 (S.D.)					
On 1 mg HGH/day					

Table 3 Influence of growth hormone on glucose, free fatty acids, and insulin during glucose infusion

		Prior to therapy	1 week	6 months	12 months	18 months	27 months
<i>Primordial growth retardation</i>							
Sch D	Glucose	195.6	244.0	284.8			
	FFA	155.4	109.2	111.4			
	IRI	2.840	4.920	5.500			
M Ch	Glucose	258.4	182.4	285.0			
	FFA	110.6	109.8	59.8			
	IRI	6.220	5.820	5.960			
A A	Glucose	254.6	173.6	212.0			
	FFA	126.4	69.2	53.4			
	IRI	11.760	11.580	11.280			
<i>Hypopituitary growth retardation</i>							
C M	Glucose	233.6		180.4	210.8	244.6	322.2
	FFA	96.2		67.6	87.4	131.4	123.8
	IRI	4.600		4.000	4.300	7.380	4.620
W D	Glucose	292.8	302.8	252.8	236.4	194.8	270.0 ^a
	FFA	73.6	127.4	136.4	62.6	100.6	85.2 ^a
	IRI	5.740	7.000	4.420	4.240	4.380	10.500 ^a
Co M	Glucose				210.6	238.0	
	FFA				86.4	98.0	
	IRI				4.760	7.980	
Co P	Glucose				211.0	218.8	
	FFA				84.6	105.8	
	IRI				9.560	9.100	
<i>Normal children</i>							
age 3-14 yrs	Glucose	215.2 ± 46.6 (n=6) (mean ± S.D.)					
	FFA	88.0 (n=2)					
	IRI	6.260 ± 3.380 (n=6)					

The values are given in min mg ml⁻¹ for glucose min μ Eq ml⁻¹ for FFA min μ U ml⁻¹ for insulin

^a On 12 mg HGH per day

area under the curve was determined planimetrically and expressed as min mg ml⁻¹ for glucose min μ Eq ml⁻¹ for free fatty acids and min μ U ml⁻¹ for insulin

Six normal prepubertal children aged 3-14 years (four boys and two girls) were tested as controls

RESULTS

The results are summarized in Table 2 and 3 and the findings in the normal children are given in each table

Effect on glucose tolerance

The K values were within the normal limits in the patients tested prior to therapy and there was no clear cut effect of growth hormone therapy on the K values. These remained in the normal range in all treated children, even in the two patients followed for 27 months. Patient W D received a six times higher dose

(12 mg HGH/day) for the last five days of the study and the K value decreased only slightly from 2.16 to 1.78

Effect on free fatty acids in plasma

Neither fasting free fatty acids (FFA) nor the decreases nor the areas under the FFA curves during and after the glucose infusion test were significantly influenced by treatment with HGH (Table 3 and Fig. 1). The higher HGH dosage in W D (Table 3) and the markedly increased endogenous GH level in K E prior to surgery (Fig. 4) were also without effect on free fatty acids in plasma.

Effect on serum insulin

In all children treated the fasting insulin concentration, the maximal rise and the area below the insulin curve during the glucose infu-

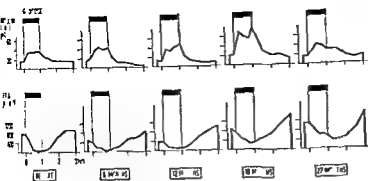


Fig 1 Influence of long term HGH (Raben) therapy (10 mg/m²/week) in a patient with hypopituitary growth retardation (C M) on serum insulin and plasma free fatty acid concentration during an intravenous glucose infusion test

mon test also remained essentially normal and unaffected by HGH therapy (Table 3 Figs 1 and 2). In patient C M a distinct increase of insulin secretion seemed to arise after 18 months of HGH therapy which however was restored to the preceding level at 27 months (Fig. 1).

Administration of the higher HGH dose in W D led to plasma GH concentrations approximately four times higher (47.3 to 102.6 µg/ml) during the test than under the ordinary dosage (11.2 to 24.0 µg/ml). Correspondingly insulin secretion was more than

twice as large under the higher dose (Fig. 3 Table 3). However it failed to reach the pathologically elevated insulin secretion found preoperatively in the acromegalic patient (K. E.) in whom plasma GH concentration was chronically elevated (18.8 to 39.8 µg/ml Fig. 4).

DISCUSSION

The value of the interpretation of a longitudinal study depends upon the reproducibility of the parameters analyzed. Repeated studies of glucose mediated insulin release under the influence of HGH in this respect are hampered by the spontaneous variability of insulin secretion after oral glucose loads from one child to another but also when repeated in the same child (27). These considerations prompted us to use standardized one hour intravenous glucose infusions which seemed more appropriate for longitudinal evaluation of the effect of long term HGH therapy on the insulin releasing capacity of the pancreas. Although variations from one subject to the other could not be avoided by this standardization the insulin release remained remarkably constant when repeated in the same individual as demonstrated graphically in Fig. 2. Both the amount of insulin released as expressed by the area below the curve and the shape of the curve remained fairly uniform in the same child in spite of growth hormone therapy.

Glucose tolerance and the effect of glucose infusion on insulin secretion and free fatty acids were essentially unaffected by HGH sub-

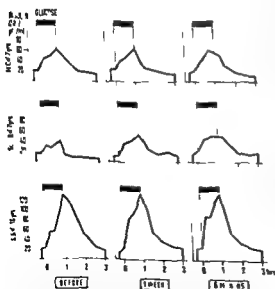


Fig 2 Influence of long term HGH (Raben) therapy (10 mg/m²/week) in three patients with primordial growth retardation on serum insulin concentration during an intravenous glucose infusion test

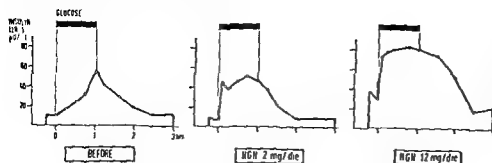


Fig 3 Influence of two different HGH doses (Raben) on serum insulin concentration during an intravenous glucose infusion test in a patient with hypopituitary growth retardation (W D)

stitution therapy in all children even after 27 months in two patients. It must however be pointed out that the effect of HGH on the initial insulin release was not tested because the first blood sample was drawn after 15 min only. Since this initial insulin peak after intravenous glucose administration is of very short duration in comparison to the sustained effect on serum insulin concentration by the prolonged glucose infusion, the result expressed as the area under the curve is not likely to be significantly falsified by disregarding the initial insulin peak. From these data, therefore, the conclusion may be drawn that chronic HGH therapy at the usual dosage must not be considered to be diabetogenic at least in children without diabetic predisposition.

Hypopituitary growth retardation may be accompanied by hypoglycemia due to lack of GH (2). Several groups of investigators, however, have pointed out recently that a diabetic glucose tolerance may also be encountered in hypopituitarism (2, 9, 10). Our two hypopituitary patients tested prior to HGH therapy (C M and W D) showed normal glucose tolerance as judged by glucose disappearance rate. The diabetic glucose tolerance found in some of the hypopituitary patients is attributed to a decrease of the glucose mediated insulin

secretion frequently found in these children prior to HGH administration (9, 10), an observation which we were able to confirm in some of our other patients with GH deficiency. However, the two patients of this study (C M and W D) showed normal insulin secretion prior to HGH therapy.

Gold et al (10) found normalization of previously subnormal insulin secretion with concomitant normalization of glucose tolerance after onset of hormonal replacement therapy. HGH, therefore, at the usual dosage seems to normalize decreased insulin secretion, without producing a pathological increase of insulin release.

Acute intravenous administration of 1 mg HGH (Wilhelmi) five minutes prior to glucose injection was reported to cause a significantly accelerated glucose utilization, probably due to an increased insulin secretion in GH deficient subjects (9). No such effect on glucose disappearance was noted in our patients when HGH (Raben) was administered intramuscularly one hour prior to glucose injection, even though the plasma GH concentrations measured in one patient (W D) were markedly elevated at the time of the test.

Preliminary estimations of the daily GH production in adults were in the order of 2-5

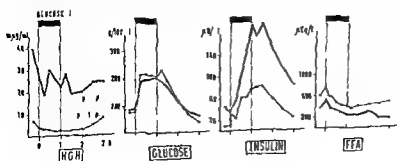


Fig 4 Effect of an intravenous glucose infusion test on serum insulin and plasma free fatty acid concentration in a patient with acromegaly (K E) before (heavy line) and three months after removal of a pituitary adenoma (thin line). Glucose tolerance remained unchanged (preoperatively $K = 0.77$, postoperatively $K = 0.73$).

mg/m (18 19) Meanwhile the study of Cameron et al (3) on the metabolic clearance rate of radiiodinated HGH revealed a multi-exponential disappearance from plasma which in fact may prevent accurate calculation of the normal daily secretion rate. The dosage of HGH substitution therapy therefore will probably remain empirical. Most investigators have used from 4 to 15 mg/week (19 20 22 25). Our dose of 10 mg/m²/week in three injections conforms with the commonly used schedule. Our observation of a lacking effect on insulin secretion is in agreement with the assumption that the usual dosage is roughly corresponding with the normal daily secretion rate. Ten times higher doses were known to evoke a pathologically increased insulin secretion in hypopituitarism (15). The present data in one of the patients (W D) revealed that a six times larger dose leads to an only slightly decreased glucose tolerance. It doubles however insulin secretion without affecting free fatty acid concentration. Our investigation does not permit to conclude whether prolonged administration of elevated HGH doses would eventually lead to exhaustion of the pancreas with permanent diabetes mellitus. It is noteworthy however that in the acromegalic patient (K E) the pathological glucose tolerance did not improve after normalization of plasma GH levels following removal of the pituitary adenoma, although there was no evidence of pancreatic exhaustion since insulin secretion was normalized. The reason for the continuing diabetic glucose tolerance in this patient in spite of normal GH and Insulin concentrations is unknown. Yet these findings indicate that chronically elevated GH concentrations may lead to irreversible diabetes mellitus. Careful adherence to the usual probably physiological HGH dosage in the treatment of hypopituitary growth retardation is therefore advocated.

SUMMARY

Daily secretion of growth hormone (GH) in the normal child is unknown and may not be

assessable in the near future. The dosage of human growth hormone (HG) commonly used in hypopituitary growth retardation is purely empirical. Growth is in general successfully accelerated by HGH but would presumably be stimulated also by higher than physiological doses. Our study consisted of a longitudinal evaluation of the effects of HGH on glucose tolerance, free fatty acids and immunoreactive insulin in three children with primordial growth retardation and two children with GH deficiency over six to twenty seven months. Two additional hypopituitary children were studied twelve and eighteen months after onset of HGH therapy. In all patients there was no discernible effect on the parameters studied at the dosage used (HGH (Raben 10 mg/m²/week in three divided injections). This finding supports the assumption that the dose of HGH commonly used is indeed physiological in regard to carbohydrate metabolism.

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Key words dwarfism growth retardation human growth hormone insulin secretion glucose tolerance free fatty acids acromegaly

STUDIES ON MATURITY IN NEWBORN INFANTS

I Birth Weight Crown-Heel Length Head Circumference and Skull Diameters in Relation to Gestational Age

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From the Department of Paediatrics University Hospital Umeå Sweden

It is now well known that birth weight is a poor index of gestational age in newborn infants and that birth weight varies over a wide range for a given gestational age. Treating infants of low birth weight (< 2500 g) as a homogeneous group has been criticized from both the clinical and scientific points of view since a high proportion of low birth weight infants are born at term (10).

The normal limits for birth weights at different gestational ages are given in standard curves for the relation between these two parameters of Hosemann (11) Lubchenko et al (15) Engstrom & Sterky (6) Sterky (20) Thomson et al (21). As a rule the relation between gestational age and crown-heel length has also been studied. Few standard curves for head circumference are available (cf Hosemann (12) Lubchenko et al (16) Usher & McLean (24) Parmelee et al (18)) the last mentioned is restricted to a material of low birth weight infants).

There is often a need for objective estimation of maturity or gestational age in the newborn infant. The fact that birth weight has been shown to be a poor index of gestational age in newborn infants has led to the introduction of other methods for estimating age in the newborn period. Postnatal radiological examination, scoring of external characteristics and other methods have been used instead of anthropometric measurements. However our

knowledge of the value of anthropometric measurements other than birth weight and crown heel length as maturity signs is incomplete and the use of such measurements ought not to be abandoned without further study.

Aims of the Present Study

- 1 To compare five anthropometric measurements (birth weight crown-heel length head circumference occipito-frontal diameter biparietal diameter) with respect to their correlation to gestational age.
- 2 To present confidence limits for estimating gestational age from anthropometric measurements of the infant.
- 3 To compare crown-heel length head circumference and skull diameters of small for gestational age infants with those of appropriate for gestational age infants.

Definitions and Abbreviations Used in the Present Study

Gestational age age in days from the first day of the mother's last menstrual period until the day of birth.

Postmenstrual age age in days from the first day of the mother's last menstrual period until the day of the investigation (i.e. gestational age plus age from birth until the investigation).

40th week days -74 to 280

SGA - small for gestational age. Infant with birth weight below -2 SD in the relation between birth weight and gestational age according to Swedish standard curves (6, 20).

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could not be classified according to the standard curves which do not consider gestational ages below 33 weeks (275 days)

The mean age of the mothers at delivery was 26 years SD 5.3 The youngest mother was 18 years the oldest 47 years III were primipara No mother had had any serious diseases during pregnancy 17 had had moderate toxemia (blood pressure above 90 mmHg diastolic in combination with albuminuria) 30 mothers had had minor bleeding at some time during pregnancy Seven infants were delivered by caesarean section the rest by vaginal delivery There were 8 breech deliveries and 2 vacuum extractions The frequencies of low fetal heart rate and low Apgar scores are seen in Table 3 as are also the most important abnormal signs during the neonatal period

In order to make a more detailed study of the small for gestational-age infants three sub-samples were selected within the investigated material

1 18 apparently healthy SGA infants (below -2 SD) All but one were full term the group is referred to as full term small for gestational age

2 A matched control group of infants with normal birth weights (between -2 and +2 SD) and of the same sexes and gestational ages as the corresponding SGA infants was selected 18 infants in all The maximal difference in gestational age between an infant in this group and the corresponding SGA infant was 7 days The mean gestational age was identical in the two groups The control group is referred to as full term appropriate for gestational age

3 Another matched control group of 18 pre term AGA infants (birth weight between -2 and +2 SD) was selected The maximal difference in birth weight between an infant in this group and the corresponding SGA infant was 40 g The mean weights in the two groups were identical In three cases it was not possible to select a pre term infant of the same sex as the SGA infant (in all three cases a boy had to be selected instead of a girl) This group is referred to as pre term appropriate for gestational age

Seven infants in the SGA group had crown-heel length below -2 SD whereas all the other infants had normal crown-heel length There were no differences between these three groups regarding age weight height parity or smoking habits of the mothers Seven mothers in the SGA group had moderate toxemia during pregnancy as compared with 3 in the pre term group and none in the full term AGA group Six of the mothers in the pre term group had bleedings during pregnancy as compared with 3 in the SGA group and none in the term AGA group

In a corresponding way a group of 9 large for gestational-age infants was selected The upper limit of the 90th percentile was chosen since only 4 infants had a birth weight above +2 SD A control group of infants of the same sexes and gestational ages (birth weights between the 10th and 90th percentiles) was selected The maximal age difference between corresponding infants in the two groups was 5 days The mean gestational age was the same for the two groups

Table 3 Important abnormal signs noticed during the perinatal period in the investigated material of 174 infants

	Number
Low fetal heart rate during delivery	13
Perinatal asphyxia (Apgar score below 7 at 1 or 10 min after birth)	26
IRDS	4
Other respiratory abnormalities	12
Obvious neurological abnormalities	5
Isommunization (anti D anti A, and other)	10
Hyperbilirubinaemia (bilirubin above 15 mg per 100 ml)	45
Minor anomalies	5

METHODS

Immediately after birth a midwife weighed the infant on a balance (Scathmos 397 Lindell AB Solna Sweden) as a part of the routine care The weight was registered and recorded to within 5 g The crown-heel length was measured by the same person using a measuring board with supports for the head and feet The length was recorded to within 0.5 cm The nearest length in whole centimetres was used in calculations Head circumference was measured on the 1st or 2nd day by the author using a tape measure The largest occipito-frontal head circumference was recorded to within 1 cm The skull diameters were measured at the same time using a caliper The values were recorded to the nearest whole centimetre

Control of methods

The weighings and measurements of birth weight and crown-heel length were controlled as follows Three nurses independently using the same instruments weighed and measured 19 infants not included in the study They were given no previous instructions in the technique Each infant was weighed by the three nurses within a short period of time thus avoiding natural variations in weight The maximal difference in the weights obtained by the three investigators was 50 g in one case 35 g in one case in the rest of the cases it was 0-25 g The mean difference was 2 g No systematic differences were seen The maximal difference in the crown-heel lengths obtained by the three investigators was 2.5 cm in one case In the other infants it was 0-1.5 cm The mean difference was 0.2 cm No systematic differences were seen

The measurements of head circumference and skull diameters was controlled in the following way The author made the measurements on 10 infants and the values were recorded A couple of hours later on the same day the measurements were repeated on the same infants, the values from the first examination not being available at the second examination There

Table 1 *Investigated material divided according to sex into gestational age groups of 2 week intervals*

Gestational age (days)	Total material	Boys	Girls
<225	6	6	0
225-238	10	7	3
239-252	18	10	8
253-266	29	15	14
267-280	42	19	23
281-294	51	22	29
>294	18	9	9
Total material	174	88	86

AGA = appropriate for gestational age Infant with birth weight within normal limits for the gestational age (between -2 and $+2$ SD or between the 10th and 90th percentiles)

LGA = large for gestational age Infant with birth weight above the 90th percentile according to Swedish standard curves

Pre term gestational age less than 267 days postmenstrual

Term gestational age between 267 and 294 days

Post term gestational age more than 294 days postmenstrual

LMP = last menstrual period

Maturity as used by the author maturity is an expression of the degree of development of the newborn infant Maturity thus defined is dependent on the gestational age of the infant but also on other factors such as biological variation (9)

MATERIALS

Criteria for selection

Only infants for whom reliable information about gestational age was available were included in the study. The mothers were interviewed by the author on one of the first days after birth. All records from the prenatal visits were checked. The following criteria had to be fulfilled for inclusion:

1 The mother could state the exact date for the beginning of her last menstrual period (LMP) at the interview. This date should be the same as that earlier given at the first antenatal visit.

2 The menstrual cycles had been regular at intervals of 28 ± 3 days before pregnancy.

3 The LMP should have been normal with respect to duration and amount and occurred on the expected date.

4 If oral contraceptives were used before pregnancy at least one spontaneous menstruation should have occurred before pregnancy.

5 There should have been no bleeding during the first 2 months after the LMP.

6 The time from the LMP to the recognition of

fetal movements should have been within the following limits for primipara: fetal movements no earlier than the 18th week and no later than the 22nd week; for multipara: no earlier than the 16th and no later than the 20th week.

Investigated material

The investigations were performed during the course of one year from November 1967 to November 1968. During this period about 270 mothers were interviewed. The above criteria were fulfilled for 187 infants who comprised the primary material. The main reason for excluding the other infants was irregular menstrual cycles. The primary material consisted of two parts. Part one included all low birth weight infants (<2500 g) born during this time at Umeå University Hospital who fulfilled the above mentioned criteria for selection. 73 infants in all. As the examination of these infants progressed the second part of the material was selected by scanning the hospital records two to three times per week for recently newborn infants with birth weights above 2500 g. The aim was to include as many infants as possible with extreme combinations of birth weight and gestational age who fulfilled the criteria for selection. In case no such infant was available a full term normal birth weight infant was studied. The second part of the material consisted of 114 infants. From the first part of the material studied 13 infants had to be excluded before analysing the results: 10 died and 1 was referred to another hospital. These infants were incompletely examined. Two infants had chromosomal aberrations. The remaining material called the *investigated material* thus consisted of 174 infants. The material divided according to sex and in gestational age groups of two week intervals is shown in Table 1. In Table 2 the infants are divided according to birth weight in groups of 500 g intervals. When the material was divided according to the relation of birth weight to gestational age (Swedish standard curves # 20) there were 19 infants below -2 SD, 145 between -2 and $+2$ SD and 4 infants above $+2$ SD. 48 infants had birth weights below the 10th percentile, 111 between the 10th and 90th percentile and 9 above the 90th percentile. Six infants

Table 2 *The investigated material divided according to birth weight into groups of 500 gram intervals*

Birth weight g	Total material
<1500	4
1501-2000	16
2001-2500	40
2501-3000	35
3001-3500	25
3501-4000	32
4001-4500	21
>4500	1

could not be classified according to the standard curves which do not consider gestational ages below 33 weeks (225 days)

The mean age of the mothers at delivery was 26 years SD 5.3 The youngest mother was 18 years the oldest 42 years 93 were primipara. No mother had had any serious diseases during pregnancy 17 had had moderate toxemia (blood pressure above 90 mmHg diastolic in combination with albuminuria) 30 mothers had had minor bleeding at some time during pregnancy Seven infants were delivered by caesarean section the rest by vaginal delivery There were 8 breech deliveries and 2 vacuum extractions The frequencies of low fetal heart rate and low Apgar scores are seen in Table 3 as are also the most important abnormal signs during the neonatal period

In order to make a more detailed study of the small for gestational-age infants three sub-samples were selected within the investigated material

1 18 apparently healthy SGA infants (below -2 SD) All but one were full term the group is referred to as full term small for gestational age

2 A matched control group of infants with normal birth weights (between -2 and $+2$ SD) and of the same sexes and gestational ages as the corresponding SGA infants was selected 18 infants in all The maximal difference in gestational age between an infant in this group and the corresponding SGA infant was 2 days The mean gestational age was identical in the two groups The control group is referred to as full term appropriate for gestational age

3 Another matched control group of 18 pre-term AGA infants (birth weight between -2 and $+2$ SD) was selected The maximal difference in birth weight between any infant in this group and the corresponding SGA infant was 40 g The mean weights in the two groups were identical In three cases it was not possible to select a pre-term infant of the same sex as the SGA infant (in all three cases a boy had to be selected instead of a girl) This group is referred to as pre-term appropriate for gestational age

Seven infants in the SGA group had crown-heel length below -2 SD whereas all the other infants had normal crown-heel length There were no differences between these three groups regarding age, weight, parity or smoking habits of the mothers Seven mothers in the SGA group had moderate toxemia during pregnancy as compared with 3 in the pre-term group and none in the full-term AGA group Six of the mothers in the pre-term group had bleedings during pregnancy as compared with 3 in the SGA group and none in the term AGA group

In a corresponding way a group of 9 large for gestational-age infants was selected The upper limit of the 90th percentile was chosen since only 4 infants had a birth weight above $+2$ SD A control group of infants of the same sexes and gestational ages (birth weights between the 10th and 90th percentiles) was selected The maximal age difference between corresponding infants in the two groups was 5 days The mean gestational age was the same for the two

Table 3 Important abnormal signs noticed during the perinatal period in the investigated material of 174 infants

	Number
Low fetal heart rate during delivery	13
Postnatal asphyxia (Apgar score below 7 at 1 or 10 min after birth)	26
IKDS	4
Other respiratory abnormalities	12
Obvious neurological abnormalities	5
Isoimmunization (anti D anti A and other)	10
Hyperbilirubinæmia (bilirubin above 15 mg per 100 ml)	45
Minor anomalies	5

METHODS

Immediately after birth a midwife weighed the infant on a balance (Statens 397 Lindell AB Lönköping Sweden) as a part of the routine care The weight was registered and recorded to within 5 g The crown-heel length was measured by the same personnel using a measuring board with supports for the head and feet The length was recorded to within 0.5 cm The nearest length in whole centimetres was used in calculations Head circumference was measured on the 1st or 2nd day by the author using a tape measure The largest occipito-frontal head circumference was recorded to within 1 cm The skull diameters were measured at the same time using a caliper The values were recorded to the nearest whole centimetre

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Table 4 Mean values for five anthropometric parameters for infants divided into gestational age groups of 2 week intervals

The values for head circumference and the two skull diameters are lacking for one infant (278 days) and the two skull diameters for one additional infant (246 days)

	<225	225-238	239-252	253-266	267-280	281-294	295-308
<i>Mean birth weight g</i>							
All infants	1 460	2 048	2 248	2 626	2 954	3 536	3 663
Boys	1 460	2 061	2 235	2 606	3 075	3 730	3 781
Girls	—	2 017	2 265	2 648	2 854	3 390	3 544
<i>Mean crown-heel length cm</i>							
All infants	41.3	45.3	46.1	47.6	48.8	50.6	51.7
Boys	41.3	45.6	46.2	47.3	49.4	51.6	52.7
Girls	—	44.7	46.0	47.8	48.3	49.8	50.7
<i>Mean head circumference cm</i>							
All infants	28.3	30.8	31.8	32.8	33.5	34.8	35.6
Boys	28.3	31.1	32.2	32.9	34.0	35.6	36.1
Girls	—	30.0	31.3	32.6	33.0	34.2	35.0
<i>Mean occipito-frontal diameter cm</i>							
All infants	10.0	10.5	10.8	10.9	11.3	11.7	12.2
Boys	10.0	10.7	11.0	11.1	11.5	12.0	12.4
Girls	—	10.0	10.6	10.8	11.2	11.4	11.9
<i>Mean bi parietal diameter cm</i>							
All infants	7.5	8.0	8.6	8.9	9.3	9.5	10.2
Boys	7.5	8.0	8.8	8.9	9.4	9.6	10.8
Girls	—	8.0	8.3	8.9	9.1	9.4	9.6
Number boys/girls	6/0	7/3	10/8 (7)	15/14	19/23 (22)	22/29	9/9

were no differences in the measurements of head circumference. For the occipito frontal diameter there was a difference of 1 cm for one child and for bi parietal diameter there was a difference of 1 cm for three infants.

All the methods used for anthropometric measurements thus gave an acceptable degree of reproducibility.

Statistical methods

The methods of sampling individuals described above are such that the characteristics (parameters) measured can be regarded as random samples.

From the statistical standpoint the aim of the present study was to obtain equations for estimating the length of gestation (Y) from other characteristics (X_1, \dots, X_n) measured. In order to obtain these relations between gestational age (Y) as dependent variable and different combinations of the other characteristics as independent variables, multiple regression analysis was used. For further details about model building see e.g. Draper & Smith (5).

There was reason to believe that many of the variables measured would give approximately the same information about gestational age. To obtain the best equation for estimating gestational age using a reduced number of variables the data were analysed by a stepwise multiple regression procedure (5) using a computer (CD 3200) and a standard program (BMD 02R version of June 2 1964 Health Sciences Com-

puting Facility UCLA). A number of different models were examined and compared. Among these the relationship between the period of gestation and the total maturity score (8) can be mentioned.

The 95% confidence limits for predicting the unknown value of Y from a new observed value (\hat{Y}) of the independent variables was calculated according to the following formula:

$$\hat{Y}_0 \pm t_{\alpha/2, n-3}^* \sqrt{\text{MSE} / (1 + \sum_{j=1}^n (X_j - \bar{X}_j)^2 / S_{xxj})}$$

where \hat{Y}_0 is the predicted value of Y , \hat{X}_j the vector value of the independent variables of the new observation and X is the matrix of the independent variables in the original sample (5, 14). The multiple correlation coefficient was also calculated.

The data for boys and girls could be compared by comparing the separate regression lines with respect to slope and position according to Brownlee (2).

For calculating the arithmetic mean and standard deviation

$$(\text{SD}) = \sqrt{\frac{\sum (Y_i - \bar{Y})^2}{n-1}}$$

conventional statistical methods were applied.

RESULTS

The mean values for the five anthropometric parameters studied, infants divided into gesta-

tional age groups of 2 week intervals are seen in Table 4 which also gives the results for boys and girls separately. There are minor differences between boys and girls in all the age groups.

In Figs 1 and 2 the values for two of the parameters birth weight and head circumference are plotted against gestational age. Boys and girls are not separated in these figures. There is a wide scatter around the linear regression line (see below) especially for birth weights in the low range. The five parameters were correlated to gestational age using linear regression analysis. The coefficients for the correlations are presented in Table 5 for boys and girls separately as well as for all infants. All correlations were significant ($p < 0.001$). The highest correlation was for head circumference. This is true for the group as a whole as well as for boys and girls separately. The linear regression equations for Y (gestational age) on X (head circumference) were

$$Y = 11.03 + 7.75 X \text{ (all infants)}$$

$$Y = -21.46 + 8.57 X \text{ (boys)}$$

$$Y = 52.54 + 6.65 X \text{ (girls)}$$

If the common formula for both sexes is used in calculating gestational age, the gestational age of boys is underestimated by about 9 days for low values and 3 days for high values of head circumference compared with the formula for

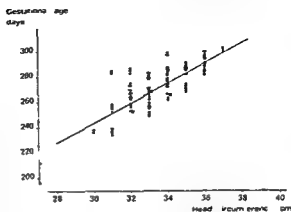


Fig 2 Head circumference plotted against gestational age. Linear regression line indicated in the figure. $N = 173$.

boys only. The gestational age of girls will be overestimated by about 10 days for low values and about 3 days for high values of head circumference. The corresponding equation for birth weight was $Y = 214.61 + 0.02 X$. Logarithmic functions did not give a significantly better expression of the relations between the five parameters and gestational age than did the linear regression analyses.

The 95% confidence limits for estimating gestational age from different values for head circumference (boys and girls together) were ± 26.5 days (mean value for infants younger than 225 days), ± 26.1 days (mean value for all infants), ± 26.1 days (mean value for infants older than 294 days). The confidence limits for birth weight (mean value) were ± 28.9 days and for crown-heel length (mean value) ± 29.7 days.

An attempt was made to analyse the anthropometric parameters together in stepwise multiple regression analysis, but only head circumference entered the multiple regression at a significant ($p < 0.05$) F value. Thus the use of a combination of anthropometric measurements did not significantly increase the precision of estimating gestational age. Analysing only birth weight and crown-heel length together gave confidence limits of ± 28.6 days.

The confidence limits for estimating gestational age from mean values of head circumfe-

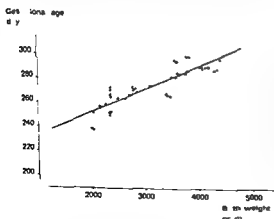


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Gestational age
days

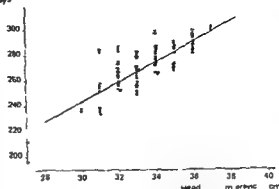


Fig 2 Head circumference plotted against gestational age. Linear regression line indicated in the figure. $N = 173$

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The confidence limits for estimating gestational age from mean values of head circumfe-

Gestational age
days

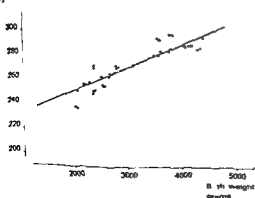


Fig 1 Birth weight plotted against gestational age. Linear regression line indicated in the figure. $N = 174$

Table 5 Coefficients for the correlation between gestational age and five anthropometric measurements

	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
All infants	0.72	0.71	0.78	0.65	0.56
Boys	0.80	0.78	0.87	—	—
Girls	0.62	0.60	0.70	—	—

Table 6 Anthropometric data, comparison between small for gestational age, pre term and full term appropriate for gestational age infants

Asterisks indicate significant differences between means

	Number	Age at birth (days)	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
Full term	18	276	2 133	46.0	31.9	10.8	8.8
SGA infants			***	***	***	**	***
Full term	18	276	3 399	49.7	34.2	11.4	9.5
AGA infants			***	***	***	***	***
Pre term	18	242	2 134	45.5	31.2	10.7	8.3
AGA infants							

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

rence for boys and girls separately were ± 23.9 days and ± 24.5 days respectively.

The mean values for the anthropometric data in 18 small for-gestational-age infants (as defined above) as compared with pre term and term AGA infants are shown in Table 6. Not only birth weight but also the other four parameters were significantly lower in SGA than in AGA infants. No significant differences were found between SGA infants and pre term AGA

infants with respect to birth weight (see principles for the selection of these two groups) but this was true also for crown-heel length, head circumference and occipito frontal diameter. Only the bi parietal diameter was significantly lower in the pre term group.

The corresponding values for nine large for gestational age infants (as defined above) and their control infants of normal birth weight is shown in Table 7.

Table 7 Anthropometric data comparison between mean values for large for gestational age infants and appropriate for gestational age infants

Asterisks indicate significant differences

	Number	Age at birth (days)	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
LGA infants	9	287	4 358	52.7	35.6	11.8	9.8
			***	**			
AGA infants	9	285	3 307	50.2	34.6	11.6	9.7

** $p < 0.01$ *** $p < 0.001$

DISCUSSION

I Estimation of gestational age from LMP

In all studies on maturity in fetuses and new born infants a crucial point is the calculation of the gestational age from the mother's information as to the LMP since the exact date of conception is rarely known. The LMP must be carefully controlled by interviewing the mother and checking the dates given at prenatal visits. The menstrual cycle must have been regular and there should have been no bleeding during early pregnancy which might have been erroneously taken for a menstruation. Ideally an early vaginal examination should be performed in order to estimate the uterine size. However this had been done in only about half of the mothers in the present study. Different values are given in the literature for the time interval between the LMP and quickening. Nonetheless recording the time when fetal movements are first noted increases the accuracy of estimation of the gestational age as recently pointed out by Rawlings & Moore (19). The time interval chosen for this study 20 ± 2 weeks for primipara 18 ± 2 weeks for multipara agrees well with the mean and standard deviation given by Rawlings & Moore. The use of oral contraceptives can postpone the time for ovulation after cessation of medication. Of 14 mothers of infants in this study who had used oral contraceptives 4 had had one spontaneous menstruation before conception the others had had 2 or more.

There is thus reason to believe that the gestational ages calculated from the information supplied by the mothers were correct and that the variations in results to be discussed below do not depend on errors in calculating gestational age from the LMP. It should be pointed out however that the interval between LMP and ovulation varies (9).

II Definitions

The definitions used have been arbitrarily chosen. As to the definition of pre- and post term delivery, an international recommendation from

1970 is to place the limit for pre term 3 weeks before term and for post term 2 weeks after term (7). It was found convenient however to divide the infants in the present study into gestational age groups of 2 week intervals thus defining pre term as 2 weeks before term. This is also in accord with suggestions of Battaglia et al (1) and others.

The definition of weeks used (i.e. 40th week = days 274-280) is in agreement with the definitions used in constructing the Swedish standard curves (6, 20) but differs from recent international recommendations (40th week = days 280-286 (7)).

SGA was defined here as birth weight below -2 SD. The two most used definitions are below -2 SD (roughly corresponding to the 3rd percentile) and below the 10th percentile. There were two main reasons for choosing the -2 SD limit. First it was considered more interesting to study a group with relatively pronounced growth retardation. Secondly it was possible within the investigated material to select matched control groups to the infants below -2 SD. This would not have been possible had the 10th percentile limit which selects many more infants been chosen.

III Results

The present material of 174 infants was selected to contain a high proportion of pre term and SGA infants. The latter fact is reflected in the lower mean weights for different gestational age groups as compared with those given in the Swedish standard curves (6, 20).

In correlating the five anthropometric parameters studied to gestational age at birth (linear regression analyses) it was found that crown-heel length did not correlate any better than birth weight to gestational age whereas head circumference had a higher degree of correlation. This is important to note since head circumference is a simple but neglected anthropometric measurement; there are few standard curves relating it to gestational age. The high correlation coefficients for head circumference as compared with the other parameters was

Table 5 Coefficients for the correlation between gestational age and five anthropometric measurements

	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
All infants	0.72	0.71	0.78	0.65	0.56
Boys	0.80	0.78	0.87	—	—
Girls	0.62	0.60	0.70	—	—

Table 6 Anthropometric data comparison between small for gestational age, pre term and full term appropriate for gestational age infants

Asterisks indicate significant differences between means

	Number	Age at birth (days)	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
Full term SGA infants	18	276	2 133 ***	46.0 ***	31.9 ***	10.8 **	8.8 ***
Full term AGA infants	18	276	3 399 ***	49.7 ***	34.2 ***	11.4 ***	9.5 ***
Pre term AGA infants	18	242	2 134	45.5	31.2	10.7	8.3

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

rence for boys and girls separately were ± 23.9 days and ± 24.5 days respectively.

The mean values for the anthropometric data in 18 small for gestational age infants (as defined above) as compared with pre term and term AGA infants are shown in Table 6. Not only birth weight but also the other four parameters were significantly lower in SGA than in AGA infants. No significant differences were found between SGA infants and pre term AGA

infants with respect to birth weight (see principles for the selection of these two groups), but this was true also for crown-heel length, head circumference and occipito frontal diameter. Only the bi parietal diameter was significantly lower in the pre term group.

The corresponding values for nine large-for gestational age infants (as defined above) and their control infants of normal birth weight is shown in Table 7.

Table 7 Anthropometric data comparison between mean values for large for gestational age infants and appropriate for gestational age infants

Asterisks indicate significant differences

	Number	Age at birth (days)	Birth weight (g)	Crown-heel length (cm)	Head circumference (cm)	Occipito-frontal diameter (cm)	Bi parietal diameter (cm)
LGA infants	9	287	4 358 ***	52.7 **	35.6	11.8	9.8
AGA infants	9	285	3 307	50.2	34.6	11.6	9.7

** $p < 0.01$ *** $p < 0.001$

is proportionally higher than the weights of most other organs (10, 17) Brain weight and head circumference are however sometimes disproportionate (17)

Parmelee et al (18) found that less than half of their infants (all low birth weight infants) with birth weights below the 10th percentile had head circumferences between the 10th and 90th percentiles. It is not stated how many of the infants with appropriate birth weight had head circumferences below the 10th percentile. The limit of -2 SD as chosen in the present study reflects a much more pronounced retardation of growth than does the 10th percentile. It is important not to draw general conclusions from comparisons between samples of SGA infants if they are not selected in comparable ways. Comparable selection means that the limits chosen (percentiles or SD) should be the same and that the standard curves used should be appropriate for the sample i.e. constructed for the type of population from which the sample is selected. The widespread use of the Denver curves (15, 16) is a source of error in this context since they were constructed from a population living at high altitude.

The large for gestational-age infants in the present material did not differ significantly from their AGA controls with respect to head circumference or the two skull diameters. However the samples were very small and thus no definite conclusions can be drawn from the results.

SUMMARY

Five anthropometric measurements: birth weight, crown-heel length, head circumference, occipito-frontal diameter and biparietal diameter were recorded in a group of 174 newborn infants of various gestational ages.

The material was selected to contain a relatively large number of infants with extreme birth weights and/or gestational ages.

Head circumference was better correlated to gestational age than were the other four measurements. Birth weight and crown heel length had the same degree of correlation to gestation

al age. The two skull diameters showed significantly lower correlations to gestational age. The confidence limits for estimating gestational age on the basis of the mean value for head circumference were $\pm 26 \text{ days}$ in this material.

In SGA infants crown-heel length and head circumference were not significantly greater than in pre term infants of the same birth weight.

ACKNOWLEDGEMENTS

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evident for boys and girls separately as well as for the group of infants as a whole. The differences in coefficients between boys and girls probably depend mainly on differences in age distribution (9). There are more boys among the pre-term infants, which is reflected in a greater standard deviation for age 240 days as compared with 169 for girls.

The occipito-frontal diameter and the bi-parietal diameter were considerably less well correlated to gestational age than was birth weight. This finding obviously casts some doubt on the value of single measurements of bi-parietal diameter in prenatal assessment of maturity, regardless of the method used, radiological examination (13) or ultrasonics (4, 3).

As can be expected from the correlation coefficients, the 95% confidence limits for estimating gestational age from anthropometric measurements were broader for birth weight than for head circumference. If anthropometric measurements are to be used in maturity assessment, head circumference is to be preferred to birth weight, crown-heel length or skull diameters. The estimation of age from head circumference alone is better than from radiological measurements of epiphyseal centres or from measuring motor conduction velocity but not as good as from evaluating external characteristics or neurological tests (9). Although the confidence limits presented here are fairly broad, largely depending on the age distribution within the sample, it can be concluded that measuring head circumference is of some practical importance in assessing maturity, especially if combined with other methods for maturity assessment (9).

The use of a combination of the anthropometric parameters studied did not significantly increase the precision of estimating gestational age, since only head circumference entered the stepwise multiple regression at significant *F*-value. This is in accord with the findings of Parmelee et al. (18) in premature infants. Timonen et al. (22) on the other hand, in a large Finnish material found that birth weight and crown-heel length together estimated gestational

age as well as did these measurements combined with head circumference. They did not state how head circumference was measured. In Scandinavia the midwives usually measure head circumference from the lowest point of the occiput to the forehead, in which case the largest head circumference is not measured. In the present material, head circumference was always given as the largest measurement.

It is well known that crown-heel length may be reduced in small-for-gestational age infants, although not necessarily to the same extent as birth weight (10). According to Usher et al. (23) the head circumference is also reduced. These authors conclude that body proportions are similar in SGA infants and pre-term infants of the same birth weight and are therefore of no value as indices of maturity. In this series there were no significant differences in crown-heel length, head circumference or occipito-frontal skull diameter between SGA and pre-term AGA infants of the same birth weight. So although head circumference is a much better index of maturity than birth weight or crown-heel length, its value in differentiating pre-term AGA and SGA infants of the same weights is limited. The definition used for SGA infants (below -2 SD) means however that only infants of markedly low birth weight were included. The SGA group in the present material was not homogeneous. Only 7 were short for gestational age as well. Excluding these 7 infants together with their respective control infants did not however significantly change the result of the comparison between the three groups.

The bi-parietal diameter on the other hand, was significantly lower in pre-term AGA infants than in SGA infants. This probably reflects the well known fact that pre-term infants often have a rather flattened head (from side to side). This difference is of limited practical use however, since the range in bi-parietal diameter is wide even at a constant gestational age. The finding of a smaller head circumference in SGA infants seems to be inconsistent with the fact that brain weight in these infants

CONTROL OF SODIUM HOMEOSTASIS IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS AND REDUCED GLOMERULAR FILTRATION RATES

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Sodium is the dominant cation in the extracellular fluid including plasma. It is thus the dominant cation in the filtrate passing the glomerular membrane. In renal insufficiency phosphate and potassium retention secondary to reduced filtration rates are common. Yet sodium retention is rarely encountered during renal insufficiency. This suggests an unusual control of sodium homeostasis even in advanced renal disease. Studies by Slatopolsky et al. have provided much information on sodium homeostasis in adult patients with renal insufficiency of various etiologies (16). In a previous series of studies we have attempted to clarify the pathophysiology of recurrent urinary tract infections in childhood (1-3). In patients with changes in the renal parenchyma demonstrated by intravenous pyelograms a wide range of functional disturbances were observed. The dominant finding was a more or less pronounced reduction of the filtration rate. This report deals with the control of sodium homeostasis in the children with recurrent urinary tract infections and a wide range of filtration rates but without clinical signs of renal insufficiency. The material studied was selected to obtain the greatest possible homogeneity in the pathophysiology. Thus

only patients with nonacute recurrent urinary tract infections uncomplicated by other renal diseases were studied. It was also thought that overlooked diseases in circulatory and hepatic organs that might influence sodium homeostasis are much more rare in children than in adults. Only children older than 6 years were studied. At that age the kidneys can be expected to work with full capacity.

The study was designed to provide information on the long time adaptation to renal disease as well as the ability to adapt to a short time oral or intravenous sodium load. Since the physiological control of sodium reabsorption and in a broader sense of sodium homeostasis and extracellular fluid volume is only partly known (4, 5, 19, 20) the interpretation of the results will have to be speculative.

MATERIAL AND METHODS

Material

17 patients aged 6 to 17 years were studied. All the patients had previous histories of urinary tract infections confirmed by urine cultures. No patient had any sign of infection during the study or at least two months prior to the study. In only two of the patients the blood urea nitrogen concentration exceeded 22 mg per 100 ml (40 and 31 mg per 100 ml respectively). Three of the patients had moderate elevation of the arterial blood pressure. An intravenous pyelo-

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Table 1 Arterial blood pressure, glomerular filtration rate during water diuresis and IVP findings in patients subjected to intravenous saline load

Patients	Blood pressure	GFR (ml/min/1.73 m ² b.s.)	Left renal parenchyma		Right renal parenchyma	
			Small in size	Uneven in shape	Small in size	Uneven in shape
SN	130/90	28	Small in size	Uneven in shape	Small in size	Uneven in shape
KH	110/70	32	Small in size	Uneven in shape	Small in size	Uneven in shape
IH	105/80	32	Small in size	Uneven in shape	Small in size	Uneven in shape
JLL	110/70	39	Small in size	Even in shape	Normal in size	Uneven in shape
LN	130/100	51	Small in size	Uneven in shape	Moderately reduced in size	Uneven in shape
IBS	125/90	82	Small in size	Uneven in shape	Moderately reduced in size	Uneven in shape
BS	110/70	96	Normal in size	Even in shape	Small in size	Uneven in shape
EJ	105/55	98	Normal in size	Uneven in shape	Normal in size	Uneven in shape
KO	100/60	104	Normal in size	Even in shape	Normal in size	Even in shape
IO	110/70	110	Normal in size	Even in shape	Normal in size	Even in shape
AE	110/65	112	Moderately reduced in size	Uneven in shape	Small in size	Uneven in shape
SA	110/55	122	Normal in size	Uneven in shape	Normal in size	Uneven in shape

gram (IVP) had been carried out in all the patients within a period of at most two years. A tabular summary of the blood pressures, glomerular filtration rates during water diuresis and the radiological findings of the patients submitted to intravenous saline loading are given in Table 1.

The studies were performed while the patients were in the paediatric metabolic ward St Gorans Hospital. All the patients were kept on a constant sodium intake 75–100 mEq/1.73 m² body surface daily 3 to 4 days before each test or hormone determination was carried out.

Studies of basal sodium excretion

The determination of the 24 hour urinary sodium excretion was generally carried out on the 3rd day of controlled diet.

Studies on the elimination of an oral sodium load

All the patients reported were submitted to this study. In order to ensure a high and constant urine flow the patients were allowed to drink water in an amount corresponding to 2–2.5% of the body weight initially and thereafter amounts slightly exceeding the diuresis every 30 min. Urine was collected by spontaneous voiding at hourly intervals. After collection of one 60 min control urine sample sodium chloride was given by mouth in a dosage of 95 mEq sodium/1.73 m² body surface. Following the administration of NaCl 5–6 urine samples were collected. Since none of the patients suffered from diarrhoea or other gastrointestinal disorders the fecal excretion was assumed to be constant and a rather insignificant factor in the elimination of the extra sodium load.

Clearance studies during water diuresis with superimposed hypotonic saline load

All the patients reported were submitted to clearance studies during water diuresis. In 12 of the patients the study was completed with a hypotonic saline load.

Water diuresis was induced in the same way as described under the oral test. When a constant diuresis had been obtained within about 60 to 90 min 3–5 clearance periods were performed. In 12 patients the clearance studies were then continued during the infusion of hypotonic saline 0.5% and 0.7% at a rate slightly exceeding the diuresis. The general proceeding for the latter part of the study was 3–5 10 min clearance periods during the infusion of 0.5% NaCl and 3–5 clearance periods during the infusion of 0.7% NaCl. The hypotonic saline was infused into the inferior vena cava catheter introduced via the femoral vein by Seldinger technique. In a few cases the studies were discontinued when the patients started to feel any discomfort such as nausea or chill. The first period was then not counted. In all the patients however the study could be continued until the saline infusion had resulted in an accumulation of 70 mEq Na⁺/1.73 m² b.s. This was generally obtained during the first periods of the infusion of 0.7% NaCl.

Standard clearance techniques were used. The glomerular filtration rate was determined by the clearance of inulin (Laevastor Gesellschaft). For urine sampling a double lumen polyethylene catheter was used enabling continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling catheter in a peripheral vein.

Clearance studies during the transition from hydropenia to water diuresis

Those studies were all performed 1–2 years prior to the salt balance studies. Nine of seventeen patients reported were subjected to this study. The protocol for these studies have been reported earlier (1). Three of the present nine patients were included in the earlier presented material.

Hormone analyses

On the fourth day of controlled diet the 24 hour

Table 2 Urinary sodium excretion ($\mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$) in patients with low and high GFRs

	Urinary sodium excretion ($\mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$)			Hydropenia versus water diuresis
	24 hour	Hydropenia	Water diuresis	
Patients with low GFRs	100 ± 26	135 ± 34	205 ± 43	$0.02 < p < 0.05$
<i>n</i>	5	4	5	
Patients with high GFRs	110 ± 37	128 ± 36	237 ± 34	$p < 0.01$
<i>n</i>	6	5	7	
Patients with low GFRs versus patients with high GFRs	$0.6 < p < 0.7$	$0.8 < p < 0.9$	$0.2 < p < 0.3$	

excretion of aldosterone 17-oxosteroids and 17 hydroxycorticosteroids were measured. Aldosterone was determined by a modification of a double isotope method described by Kluman & Peterson (9). 17-oxosteroids and 17 hydroxycorticosteroids were determined spectrographically (2).

Chemical analyses

Sodium and potassium concentrations in serum and urine were determined by a flame photometer. Osmolality cryoscopically with a Knauer microosmometer and inulin according to the method of Heyrovsky (7).

For the statistical calculation Student's *t* test has been used.

RESULTS

Glomerular filtration rate

Depending upon the values for the glomerular filtration rates (GFRs) the patients could be divided into two groups: (a) patients with severely to moderately reduced (low) filtration rates and (b) patients with subnormal to normal (high) filtration rates. During water diuresis the filtration rates were $36.4 \pm 9.1 \text{ ml}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ and $103.4 \pm 12.9 \text{ ml}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ for the two groups respectively. Mean values \pm one standard deviation are given.

Basal sodium excretion

The total urinary sodium excretion for the patients with low GFRs and the patients with high GFRs are given in Table 2. The values are obtained from 24 hour urine specimens from clearance studies during hydropenia and from clearance studies during water diuresis. The 24 hour urinary sodium excretion averaged $100 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ in the patients with

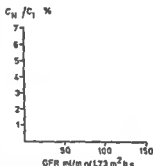


Fig. 1 The relationship between glomerular filtration rate ($\text{ml}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$) and fractional sodium excretion (C_w/C_i). The values were obtained during water diuresis.

low GFRs and $110 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ in the patients with high GFRs. The difference between the two groups was not significant. The urinary sodium excretion during hydropenia was $135 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ in the patients with low GFRs and $128 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ in the patients with high GFRs. The difference between the two groups was not significant.

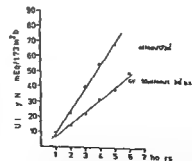


Fig. 2 The cumulative sodium excretion each hour following an oral sodium load in a patient with glomerular filtration rate of $112 \text{ ml}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$ and in a patient with glomerular filtration rate of $50 \text{ ml}/\text{min}/1.73 \text{ m}^2 \text{ b.s.}$

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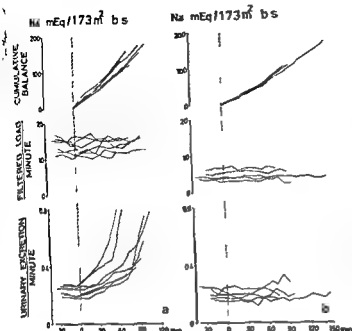


Fig 4 The effect of hypotonic saline infusion on cumulative sodium balance, filtered load of sodium and urinary sodium excretion in patients with high GFRs (a) and in patients with low GFRs (b). The values to the left of the dashed line are obtained during water diuresis alone. The dashed line shows the time when the hypotonic saline load was started (0 min).

saline load in patients with low GFRs. In contrast, in patients with high GFRs, hypotonic saline load resulted in a significant depression in the amount of reabsorbed sodium and thus a significant increase in fractional sodium excretion.

Renal response to transition from hydropenia to water diuresis

Table 4 demonstrates the values for glomerular filtration rates, filtered sodium, reabsorbed sodium, urinary sodium excretion and fractional

Table 3 Changes in glomerular filtration rate, filtered, reabsorbed and excreted sodium when sodium balance is increased 70 mEq/1.73 m² b.s. during water diuresis

	GFR (ml/min/ 1.73 m ² b.s.)	Filtered Na ⁺ (μEq/min/ 1.73 m ² b.s.)	Reabsorbed Na ⁺ (μEq/min/ 1.73 m ² b.s.)	U _{Na} V (μEq/min/ 1.73 m ² b.s.)	C _{Na} /C _{Cr} ()
Patients with low GFRs					
n	5	5	5	5	5
Mean value during water diuresis ± one standard deviation	36.4 ± 9.1	4.849 ± 1.214	4.644 ± 1.220	205 ± 48	4.46 ± 1.64
Difference of mean water diuresis versus accumulated balance of + 70 mEq Na ⁺ /1.73 m ² b.s.	0.4 0.3 < p < 0.4	-5 p > 0.9	-2 p > 0.9	-3 p > 0.9	-0.11 p > 0.9
Patients with high GFRs					
n	7	7	7	7	7
Mean value during water diuresis ± one standard deviation	103.4 ± 12.9	11.600 ± 1.909	13.362 ± 1.933	237 ± 34	1.82 ± 0.50
Difference of mean water diuresis versus accumulated balance of + 70 mEq Na ⁺ /1.73 m ² b.s.	-0.3 0.7 < p < 0.8	-81 0.3 < p < 0.4	-260 0.02 < p < 0.05	181 p = 0.05	1.45 0.02 < p < 0.05

cant. The difference in urinary sodium excretion between the 24 hour sampling and the hydro-penic sampling is not significant. During water diuresis there is a significant increase in urinary sodium excretion in both groups. The rise in urinary sodium excretion was somewhat higher in the patients with high GFRs but the difference between the two groups during water diuresis was not statistically significant. Thus the excreted amount of sodium found in 24 hour urine specimen as well as during sampling in hydropenia and water diuresis was apparently independent of the glomerular filtration rate. To effect this the fraction of filtered sodium excreted must rise when the filtration rate is reduced. This is illustrated in Fig. 1 where the fractional sodium excretion during water diuresis has been related to the glomerular filtration rate.

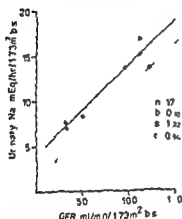


Fig. 3 The relationship between the glomerular filtration rate (ml/min/1.73 m² bs) and the urinary elimination rate (mEq/hr/1.73 m² bs) of an orally administered sodium load. All patients studied are included. The values for glomerular filtration rate were obtained during water diuresis. The urinary sodium elimination rate has been calculated by plotting cumulative sodium excretion against time (Fig. 2). *n*: Number of patients; *b*: regression coefficient; *s*: standard deviation; *r*: correlation coefficient. The solid line represents the line of regression; the dashed lines ± 2 standard deviations.

Renal response to oral sodium load

Fig. 2 demonstrates the rate of urinary elimination of an oral sodium load in a patient with high GFR and in a patient with low GFR. The accumulated amount of sodium that has been

excreted from the time when the last tablet was given has been plotted against time. A more rapid sodium elimination in the patient with high GFR was a characteristic finding. Fig. 3 demonstrates the highly significant ($p < 0.01$) correlation coefficient (0.94) between the slope of the sodium excretion line and the filtration rate.

Renal response to superimposed hypotonic saline load during water diuresis

Fig. 4a and b demonstrate the rate of sodium accumulation, the filtered load of sodium and the total urinary sodium excretion in all patients during hypotonic saline loading. The patients with high GFRs are represented in Fig. 4a and the patients with low GFRs in Fig. 4b. The filtered load of sodium fluctuated somewhat probably as a consequence of noncharacteristic changes in the GFR. In the patients with high GFRs the urinary sodium excretion started to increase continuously already when the accumulated changes in sodium balance exceeded 30 mEq/1.73 m² bs. When the accumulated changes exceeded 70 mEq/1.73 m² bs the rise in urinary sodium excretion became even steeper. In contrast the patients with low GFRs demonstrated no characteristic change in urinary sodium excretion when the sodium balance was progressively increased to 70–100–180 mEq/1.73 m² bs.

Table 3 demonstrates a statistically analysed summary of the effects of the intravenous hypotonic saline load on the renal handling of sodium in all the patients studied. The values obtained in water diuresis alone have been compared to those obtained when a positive sodium balance of 70 mEq/1.73 m² bs was achieved. No significant change in the glomerular filtration rate was seen in either of the two groups. As a consequence of the relatively stable GFR the filtered sodium load did not change significantly in either group following the superimposition of the saline load. In patients with low GFRs the amount of reabsorbed sodium did not change. Thus the fractional sodium excretion was not affected by hypotonic

Table 5 24 hr urinary excretion of aldosterone 17 oxosteroids and 17 hydroxysteroids in 10 of the patients subjected to intravenous saline loading

Patients	Aldosterone (μ g/24 hr)	17 oxo- steroids (mg/24 hr)	17 hydroxy corticosteroids (mg/24 hr)	
SN male 11 $\frac{1}{2}$ years	21.4	15	20	
KH female 16 $\frac{1}{11}$ years	24.8	168	21.5	
IH female 17 $\frac{7}{11}$ years	18.6	14.1	20.5	
ILL female 12 $\frac{1}{11}$ years	12.8	5.5	18.3	
LN female 11 $\frac{6}{11}$ years	10.0	5.9	11.8	
JBS female 9 $\frac{1}{11}$ years	6.4	2.0	5.7	
BS female 9 years	19.2	3.6	16.6	
IO female 11 $\frac{1}{11}$ years	11.8	2.3	11.3	
AE female 14 $\frac{1}{11}$ years	12.5	5.9	15.3	
SA female 9 $\frac{1}{11}$ years	5.4	2.2	3.9	
	9-12 years	12-14 years	14-15 years	15-17 years
Aldosterone normal values μ g/24 hr	5	5	5	5
17-oxosteroids normal values mg/24 hr mean	15	25	45	75
range	0.2-3.0	0.8-5.0	1.8-7.8	3.5-11.0
17 hydroxycorticosteroids normal values mg/24 hr mean	3.8	5.0	5.7	8.0
range	1.6-7.4	2.0-8.6	3.5-11.0	5.0-12.0

not been calculated before. The intravenous saline load in the previous reports were apparently started at a lower level of extracellular fluid volume and the effect i.e. the inhibition of sodium reabsorption might have been the same effect that was observed during the transition from hydropenia to water diuresis in the present study.

There are several possible explanations for the basal reset of glomerular tubular balance and thus increased fractional sodium excretion in the patients with low GFRs. Since none of the patients were uremic an osmotic diuresis due to retention of poorly reabsorbable solutes can be ruled out.

The finding of normal or increased aldosterone excretion and normal 17-oxosteroid and 17 hydroxycorticosteroid excretions practically excluded a decreased stimulation of those hormones.

A damage of the renal parenchyma might explain the increased fractional sodium excretion by at least two possible mechanisms. (a) disease of the renal tubule could result in a depression of the active reabsorptive capacity for sodium. Experimental studies have how-

ever demonstrated that renal disease is not a prerequisite for the reset of glomerular tubular balance for sodium. Thus reset of glomerular tubular sodium balance is also observed in the intact remaining nephrons when the rest of the renal parenchyma is out of function by unilateral nephrectomy and contralateral renal infarction (15). (b) If the nephron population is reduced and the remaining nephrons are hyperperfused an increase in fractional sodium excretion might also occur. This assumes that the amount of sodium and water perfusing individual remaining nephrons will exceed the reabsorptive capacity for sodium. Evidence against this hypothesis are presented by the results of the studies during transition from hydropenia to water diuresis. The patients with low filtration rates were then able to increase the reabsorptive capacity for sodium despite simultaneous increases in filtration rate i.e. nephron perfusion rate.

Another and perhaps more attractive explanation is that the reset in glomerular tubular balance during low filtration rate is a product of the physiological control mechanisms governing sodium reabsorption. The importance of

Table 4 Changes in glomerular filtration rate, filtered, reabsorbed and excreted sodium during the transition from hydropenia to water diuresis

	GFR (ml/min/ 1.73 m ² b.s.)	Filtered Na ⁺ (μEq/min/ 1.73 m ² b.s.)	Reabsorbed Na ⁺ (μEq/min/ 1.73 m ² b.s.)	U _{Na} V (μEq/min/ 1.73 m ² b.s.)	C _{Na} /C _{in} ()
Patients with low GFRs					
n	4	4	4	4	4
Mean value during hydropenia ± one standard deviation	34.0 ± 15.6	4.794 ± 2.142	4.660 ± 2.115	135 ± 34	3.04 ± 0.61
Difference of mean hydropenia versus water diuresis	13.3 p < 0.01	1.606 p < 0.01	1.477 0.01 < p < 0.02	129 0.01 < p < 0.02	1.15 0.05 < p < 0.1
Patients with high GFRs					
n	5	5	5	5	5
Mean value during hydropenia ± one standard deviation	95.0 ± 11.1	12.992 ± 1.517	12.864 ± 1.490	128 ± 36	0.98 ± 0.19
Difference of mean hydropenia versus water diuresis	13.7 0.02 < p < 0.05	1.633 0.05 < p < 0.1	1.523 0.05 < p < 0.1	110 p < 0.01	0.64 0.01 < p < 0.02

sodium excretion during transition from hydropenia to water diuresis in the patients with low GFRs and in the patients with high GFRs. A significant rise in glomerular filtration rate during the transition from hydropenia to water diuresis was found in both groups. As a consequence of this there is also a rise in the amount of filtered sodium which is significant in the patients with low GFRs and borderline significant in the patients with high GFRs. The amount of reabsorbed sodium is significantly increased in the patients with low GFRs and borderline significantly increased in the patients with high GFRs. The urinary sodium excretion was significantly increased in both groups. The fractional sodium excretion increased with borderline significance in the patients with low GFRs and with full significance in the patients with high GFRs.

Urinary steroid excretion

The 24 hour excretion of aldosterone, 17-oxosteroids and 17-hydroxycorticosteroids in 10 of the patients subjected to intravenous saline loading are given in Table 5. The excretion of aldosterone was increased or on the upper limit of the normal values for age given by Visser (18). The excretion of 17-oxosteroids and 17-

hydroxycorticosteroids were normal or slightly increased as compared to previous reported values for healthy children in the same age group (10).

DISCUSSION

The control of sodium homeostasis during low glomerular filtration rate has previously been studied during conditions of experimental uremia in dogs where unilateral nephrectomy and 85% infarction of the contralateral kidney had been carried out (15). It has also been studied in a group of patients with uremia of various etiologies including pyelonephritis, glomerular nephritis and polycystic kidneys (16). The present observation of an increased fractional sodium excretion and thus a basal reset of glomerular tubular balance for sodium at low levels of GFR is in accordance with those previous studies. The present finding of a reduced ability to rapidly adjust to changes in sodium balance in patients with low GFRs is however somewhat in contrast to what has been reported previously. This apparent difference might however only be due to variations in experimental design. Thus when testing the effect of oral sodium load the elimination rate of sodium has

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Patients	Aldosterone ($\mu\text{g}/24 \text{ hr}$)	17 oxo- steroids ($\text{mg}/24 \text{ hr}$)	17 hydroxy corticosteroids ($\text{mg}/24 \text{ hr}$)	
SN male 11 $\frac{1}{2}$ years	21.4	1.5	2.0	
KH female 16 $\frac{1}{2}$ years	24.8	16.8	21.5	
IH female 17 $\frac{1}{2}$ years	18.6	14.1	20.5	
ILL female 1 $\frac{3}{4}$ years	12.8	5.5	18.3	
LN female 11 $\frac{1}{2}$ years	10.0	5.9	11.8	
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SA female 9 $\frac{1}{2}$ years	5.4	2.2	3.9	
	9-12 years	12-14 years	14-15 years	16-17 years
Aldosterone normal values $\mu\text{g}/24 \text{ hr}$	5	5	5	5
17-oxosteroids normal values $\text{mg}/24 \text{ hr}$ mean	1.5	2.5	4.5	7.5
range	0.2-3.0	0.8-5.0	1.8-7.8	3.5-11.0
17 hydroxycorticosteroids normal values $\text{mg}/24 \text{ hr}$ mean	3.8	5.0	5.7	8.0
range	1.6-7.4	2.0-8.6	3.5-11.0	5.0-12.0

not been calculated before. The intravenous saline load in the previous reports were apparently started at a lower level of extracellular fluid volume and the effect, i.e. the inhibition of sodium reabsorption might have been the same effect that was observed during the transition from hydropenia to water diuresis in the present study.

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Another and perhaps more attractive explanation is that the reset in glomerular tubular balance during low filtration rate is a product of the physiological control mechanisms governing sodium reabsorption. The importance of

physical forces in the control of sodium reabsorption is well established (11, 17). Thus changes in peritubular capillary hydrostatic and oncotic pressure and in interstitial volume will immediately effect sodium reabsorption. No measurement of extracellular fluid volume or plasma volume was made in the present material but there were no clinical signs of fluid retention in the patients with low filtration rates. Two of five patients with low GFRs (SN and LN) had only moderate elevations of blood pressure. The remaining three patients with low GFRs had normal arterial blood pressure. None had hypoproteinemia. Thus physical factors do not appear to be of primary importance in the basal reset of glomerular tubular balance.

Evidence has also been presented for the existence of a natriuretic factor that would adjust to changes in sodium and fluid homeostasis by affecting the tubular sodium reabsorption (4-6, 13, 14). Although definite evidence for the existence of this additional factor is still lacking, the presence of several factors regulating sodium and fluid homeostasis does not seem unlikely. One of the important obstacles to the natriuretic factor theory is the lack of a common denominator for the various precipitating factors suggested: reduced filtration rate, extracellular fluid expansion and positive sodium balance. Possibly an increased demand of oxygen (decreased perfusion, increased work) might be a driving force in the release of natriuretic factor. The present results are compatible with the existence of an additional natriuretic factor that monitors the basal reset of glomerular tubular balance for sodium during low filtration rate.

When the ability to eliminate a rapidly induced positive change in sodium balance was tested the patients with low filtration rates demonstrated a significantly slower sodium excretion than the patients with high filtration rates. This was found following both oral and intravenous loads of sodium. When an oral load of sodium was given the elimination rate could be directly correlated to the filtration rate. The oral test was thought to be the most physio-

logical stimulus of the factors controlling sodium homeostasis. It was also found to be an easily accomplished and valuable clinical test for the control of sodium homeostasis.

The intravenous studies enabled determinations of absolute values of filtered and reabsorbed sodium as well as of fractional sodium excretion. Thereby more information could be obtained on the nature of the slow sodium elimination in patients with low filtration rates. The patients with high GFRs respond to the hypotonic saline load with an inhibition of tubular sodium reabsorption. This response is the same that has been observed previously in healthy experimental animals (12). The patients with low filtration rates were unable to decrease the tubular sodium reabsorption during the saline load. The slow excretion of sodium load in patients with low filtration rates could thereby be directly attributed to an inability to further suppress tubular sodium reabsorption. In contrast to the response to a hypotonic saline load the transition from hydropenia to water diuresis elicited an increase in the urinary sodium excretion in the patients with low filtration rates. This was effected not only by the increase in filtered sodium but also by a borderline sig-

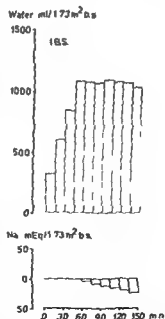


Fig 5 The accumulated changes in water and sodium balance during the transition from hydropenia to water diuresis in patient 1BS.

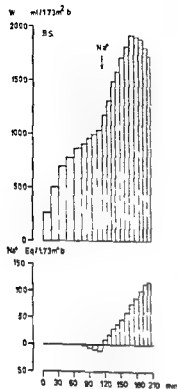


Fig 6 The accumulated changes in water and sodium balance during the course of a hypotonic saline load in experiment in patient BS

nificant increase in fractional sodium excretion. Thus the transition from hydropenia to water diuresis must have initiated an inhibition of sodium reabsorption in patients with low GFRs. During the transition from hydropenia to water diuresis the following changes are accomplished (a) a loss of the effect of antidiuretic hormone (ADH) (b) a positive water balance is induced with insignificant or negative changes in sodium balance (Fig 5). The loss of ADH effect as such could hardly be expected to cause an increase in urinary sodium excretion since in fact the addition of vasopressin itself during water diuresis results in natriuresis (8). Thus it is likely that extracellular volume expansion alone is a stimulus to inhibition of sodium and water reabsorption in patients with low GFRs. During superimposed hypotonic saline load the extracellular volume is further expanded and in addition the sodium balance is changed from slightly negative to positive (Fig 6). The lack

of further inhibition of sodium reabsorption during superimposed hypotonic saline load might be due to (a) the ability to respond to extracellular volume expansion alone is reduced in the patients with low filtration rates as compared to the patients with normal filtration rates and has already reached its maximum during water diuresis and (b) the induction of a positive sodium balance in the patients with high GFRs released an additional factor controlling sodium reabsorption. In the patients with low filtration rates this factor was either not released or more likely had been released maximally during basal conditions. The present data do not allow any further differentiation between those theories.

SUMMARY

Basal sodium excretion and rapid response to oral and intravenous sodium loads have been studied in children with recurrent urinary tract infections and a wide range of glomerular filtration rates. Basal sodium excretion related to body surface was remarkably stable in all children studied and thus independent of glomerular filtration rate. The rapid response to an oral or an intravenous sodium load was reduced in patients with low filtration rates. The urinary sodium excretion rate following oral sodium load was correlated to the glomerular filtration rate with high statistical significance. In patients with high filtration rates saline infusion resulted in a rather prompt inhibition of tubular sodium reabsorption with consequent increase in urinary sodium excretion. In patients with low filtration rates inhibition of tubular sodium reabsorption following intravenous saline load was much less pronounced. During the transition from hydropenia to water diuresis without extra sodium supply both the patients with low and with high filtration rates increased the absolute as well as the fractional sodium excretion concomitantly with an increase in the filtered load of sodium. The results are compatible with but do not prove the existence of

an additional natriuretic factor that keeps the basal sodium excretion constant in the diseased kidney

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We are greatly indebted to Hans Loow Ph D for carrying out the steroid analysis Miss Lill Britt Jönsson Miss Eivor Sundqvist and Mrs Britt Söderqvist provided expert technical assistance throughout the whole study

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THE INFLUENCE OF OROTIC ACID ON THE SERUM BILIRUBIN LEVEL OF MATURE NEWBORN

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By the application of enzyme inductors we succeeded in effectively decreasing the serum bilirubin level of premature and mature infants and in counteracting the development of a hyperbilirubinemia. Unfortunately however the inductors so far tested have certain disadvantages that prohibit their clinical application or would restrict it. The application of phenobarbital (4, 17, 19, 20, 22) is not indicated in premature infants with disorders of respiration because of its depressive effects on respiration (10). The application of phenyl butazone (5) causes edema to develop. Moreover a displacement of the bilirubin from its protein bond in the serum is likely to occur (1) so that its clinical administration cannot be advised. The effect of a therapeutically justifiable dose of nikethamide is too small to achieve a decisive clinical advantage (11).

There are indications that in addition to a not yet matured glucuronyl transferase activity which can be stimulated by inductors a deficiency of the pertinent coenzyme UDP also limits the formation of glucuronyl in the newborn child (14, 15, 23, 24). It was pointed out by Brodersen (2) that the synthesis of the UDP takes place via the phase of the orotic acid and that an increased formation of the coenzyme is perhaps achieved by the supply of this biogenic substance. In this way an increase in the bilirubin conjugation would be possible.

Setting out from this conception we at first tried to decrease the serum bilirubin level in premature infants by the application of orotic acid (6, 12). From the clinical point of view as well as for theoretical considerations we took an interest in the question whether the effect of orotic acid in decreasing bilirubin would likewise be detectable in the mature newborn child. We therefore carried out adequate investigations the results of which are submitted in this paper.

METHOD

At a neonatal unit consisting of four wards 52 mature newborn in two wards were given 200 mg of orotic acid (trial product of VEB Jenapharm Jena) orally in two single doses daily from their 1st to their 5th day of life. Fifty newborn children in the other two wards within the same test period served as a control group. The average birth weight of those of the newborn who were treated with orotic acid was 3 470 g and of the newborn from the control group 3 465 g.

We included in the test and control groups only clinically inconspicuous babies whose mothers had not been given any medication during parturition. No children from rhesus immunized mothers were included in either the test group or the control group. In both groups the newborn were first given mother's milk 6 hours after birth; the quantity of milk then increasing daily by 50 ml. On their first 3 days of life an additional feeding of a tea glucose mixture up to a total quantity of 200 g was administered. In cases of hypogalactia the feeding was done in the same mode with pooled mother's milk from the lactarium.

From the 3rd till the 5th day of life the determination of the total and indirectly reacting bilirubin

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and decrease of the serum bilirubin level whereas its administration to premature infants did not. We came to the same conclusion after increasing the daily dose of orotic acid to 600 mg. Matsuda & Shirahata (16) administered a daily dose of 200 mg of orotic acid and found only a slight decrease of the serum bilirubin level in 16 mature infants in comparison with a control group.

As to the mode of action of the orotic acid we assume a substitution effect on the coenzyme of the glucuronyl transferase. We should like to interpret our results as an indication of the fact that this substitution effect becomes effective only in premature infants with a decreased concentration of glucuronic acid. No influence can be observed in mature infants with an approximately normal level of glucuronic acid. Only in the condition where the not yet fully developed glucuronyl transferase activity of these mature infants might be developed to its full efficiency by enzyme induction could a consequent relative deficiency of coenzyme possibly be remedied by administration of orotic acid.

We are presently examining this question in connection with a combined administration of enzyme inductor and orotic acid to mature newborn infants.

It is a matter for discussion whether an enzyme inducing effect should be attributed to the orotic acid itself. This possibility is disproved by electron microscopical examinations of the liver in the course of which no increase in the endoplasmic reticulum was detectable after administration of orotic acid as well as by the determination of the liver dependent coagulation factors the activity of which showed no increase during administration (7). Animal experiments are in agreement with these results (13). After all the results given in this paper have to be assessed in a like manner. If orotic acid possessed an inductor property it would also have to be detectable in the mature newborn child as in known of other enzyme inducers.

The question remains open whether or not

the orotic acid has a choleretic effect in the premature infant. On the one hand there are indications that the elimination of bilirubin from the liver cell into the gall is decreased in the premature infant (21) on the other hand a choleretic effect of orotic acid was found by Charbonnier & Sagon (3). The absence of any influence on the bilirubin levels can be taken as proof that there is no such effect in mature infants.

We did not observe any side effects of the orotic acid in mature infants nor were these to be expected on account of our examinations carried out with premature infants (7).

SUMMARY

Fifty two mature newborn were treated with a daily dose of 200 mg of orotic acid from their 1st to their 5th day of life. An equally large number of untreated children served as a control group. Contrary to the premature infants in the mature newborn no decrease in the serum bilirubin was achieved by administration of orotic acid. The question of the possible mode of action of the orotic acid is discussed.

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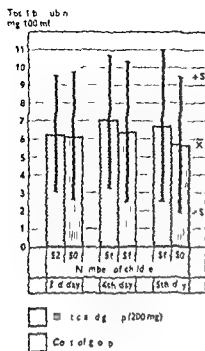


Fig 1

was carried out after the method of Jendryasik et al (8, 9)

RESULTS

Arithmetical average value and standard deviation of the total and the indirectly reacting serum bilirubin were calculated from the 3rd till the 5th day of life. As Figs 1 and 2 show

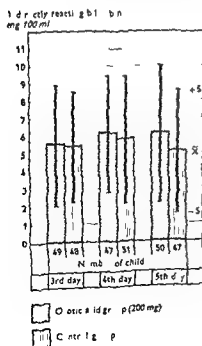


Fig 2

Table 1 Number of blood exchange transfusions in premature infants after administration of a daily dose of 100 mg respectively 300 mg of orotic acid in comparison with the control group

	Number of premature infants	Number of blood exchange transfusions	Significance (χ^2 test)
Control group	54	15	
100 mg of orotic acid daily	46	6	$p=5$
Control group	102	30	
300 mg of orotic acid daily	102	4	$p=0.1$

the average values of the serum bilirubin levels of the newborn who were treated with orotic acid are slightly higher than in the control group, but there is no significant difference. Blood exchange transfusions were not necessary in either group. The bilirubin levels of children that had an existing OA- or OB blood group constellation were watched separately. In comparison with the other newborns no higher serum bilirubin levels were registered.

DISCUSSION

We administered a daily dose of 100 mg of orotic acid to premature infants from the 1st till the 6th day of life and were thus able to decrease the number of blood exchange transfusions remarkably (Table 1) (6). The bilirubin decreasing effect was even more significant after an increase in the daily dose of orotic acid up to 300 mg. The number of blood exchange transfusions became about a tenth of that of the control group (Table 1) (12). Further investigations are being made to decide the question whether an additional increase in the daily dose of orotic acid is justifiable and whether the bilirubin decreasing effect in the premature child could be further increased in this way.

The results of the investigations submitted in this paper show that the application of orotic acid in mature infants did not lead to

a decrease of the serum bilirubin level whereas its administration to premature infants did not. We came to the same conclusion after increasing the daily dose of orotic acid to 600 mg. Matsuda & Shirahata (16) administered a daily dose of 200 mg of orotic acid and found only a slight decrease of the serum bilirubin level in 16 mature infants in comparison with a control group.

As to the mode of action of the orotic acid we assume a substitution effect on the coenzyme of the glucuronyl transferase. We should like to interpret our results as an indication of the fact that this substitution effect becomes effective only in premature infants with a decreased concentration of glucuronic acid. No influence can be observed in mature infants with an approximately normal level of glucuronic acid. Only in the condition where the not yet fully developed glucuronyl transferase activity of these mature infants might be developed to its full efficiency by enzyme induction could a consequent relative deficiency of coenzyme possibly be remedied by administration of orotic acid.

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REVIEW ARTICLE

ADHESIONS OF LABIA MINORA (SYNECHIA VULVAE) IN CHILDHOOD

A Review and Report of Fourteen Cases

EDEL HARLUND CHRISTENSEN and JAKOB ØSTER

From the Department of Paediatrics Centralsygehuset Randers Denmark

The terms adhesions of the labia minora or "synechia vulvae" denote a condition of complete or partial fusion of the labia minora in the midline. The condition has been long recognized and previously considerable interest was shown for it (3-8). In recent years, however, only superficial mention has been made of the condition even in recognized textbooks of paediatrics (17-20) and it has also received only scant mention in paediatric journals (4, 12, 14, 23, 25).

In the majority of cases the condition does not produce any symptoms but occasionally difficulties in micturition and urinary infections may occur. Early diagnosis ensures that treatment which is very easy may be undertaken before the patient has experienced somatic or mental repercussions and before the parents for whom it may be a great psychological trauma to discover that their daughter is "abnormal" are exposed to unnecessary anxiety. A number of parents and several doctors have in fact erroneously suspected girls with this condition of being hermaphrodites. This may best be illustrated by quoting Campbell's example (7). He was consulted by a mother with two daughters aged 5 and 6 years respectively both of whom had vulval adhesions. The mother had consulted several doctors and had been told

that plastic vaginal reconstruction would be necessary. In desperation the mother had made a list of 11 distinguished plastic surgeons from all over USA and intended to consult them all in order to hear about their methods of treatment. Campbell immediately undertook simple separation of the labia minora which revealed that both of the girls had normal external genitalia and normal vaginae.

It is therefore considered justifiable to draw attention to the condition and to emphasize the significance of thorough investigation of the genital region in girls not only at routine health examinations of children by the general practitioner but also at objective investigations during hospitalization.

The authors' cases

During the 5 year period 1966-70 the authors have observed and treated 14 girls with adhesions of the labia minora. Seven of the patients were admitted on account of other conditions and the vulval adhesions were discovered at routine objective investigation. The remaining 7 cases were found by a general practitioner (F. Hesselbjerg) at routine medical examinations of healthy children and referred for outpatient observation and treatment as he was aware of the authors' interest in this condition.

The age distribution of the patients appears in Table 1 which also reveals the extent of the adhesions and other clinical data. The various forms of adhesions are illustrated in photographs 1, 2 and 3.

All of the patients were symptom free. In particular

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that plastic vaginal reconstruction would be necessary. In desperation the mother had made a list of 11 distinguished plastic surgeons from all over USA and intended to consult them all in order to hear about their methods of treatment. Campbell immediately undertook simple separation of the labia minora which revealed that both of the girls had normal external genitalia and normal vaginae.

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The age distribution of the patients appears in Table 1 which also reveals the extent of the adhesions and other clinical data. The various forms of adhesions are illustrated in photographs 1, 2 and 3.

All of the patients were symptom free. In particular

Table 1 Clinical aspects of adhesions of the labia minora in fourteen cases

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age at observation (months)	5	5	6	8	8	12	14	16	17	17	23	24	48	72
Complete adhesion	+	+	+	-	-	-	-	+	-	+	-	-	+	+
Partial adhesion	-	-	-	-	+	+	-	-	+	-	+	+	-	-
Multiple adhesion	-	-	-	+	-	-	+	-	-	-	-	-	-	-
Relapse	-	-	-	-	-	-	-	-	-	-	+	-	+	-

there were no difficulties in micturition no urinary infections nor evidence of vulvitis.

Treatment consisted of rupture of the adhesions by means of slight manual traction without anaesthesia. The two oldest girls aged 4 and 6 years did not cry or complain during this therapeutic procedure while the smaller girls occasionally cried briefly. The impression was obtained that the process could not have been gentler under local or general anaesthesia and that these were not necessary. In a single case (Case 8) no treatment was undertaken during hospitalization but the mother was instructed to lubricate the synechial membrane with an indifferent ointment. During this procedure the mother perforated the membrane by accident and there has been no recurrence.

Following manually produced rupture of the synechial membrane an epithelial defect of the inner surface of the labia minora could be observed but no haemorrhage occurred in any of the 14 cases. The mothers were instructed to apply an indifferent ointment to the wound edges thrice daily for a week and thereafter to inspect and wash the external genitalia duly in the subsequent months.

All of the patients were followed up in view of possible recurrence after periods of observation which varied from 1½ to 24 months (average 7½ months) after treatment. Recurrence occurred in two girls and as far as could be judged this was because the mother had not carried out the above mentioned therapeutic programme.

DISCUSSION

1 Pathological anatomical conditions

Fusion between the labia minora consists of an extremely thin, transparent membrane which is commonly thicker posteriorly. According to Finlay (10) the adhesion not only consists of fusion of the two adjacent edges of the labia minora but frequently involves the inner surface of the labia. This was also observed in the 14 patients of this material in whom the membrane was in all of the cases and in its entire extent, so thin that suitable lateral traction sufficed to complete the rupture.

According to several authors (7, 15) the most frequent form is the complete adhesion in which fusion ceases a few millimetres posterior to the clitoris but anterior to the external urethral meatus so that an opening remains through which micturition can occur. In occasional cases one or two openings may be present posteriorly in the membrane. Nowlin et al (15) observed conditions such as these in 47% out of their 110 patients. Finally, adhesions may be partial and in such cases frequently corresponding to the posterior 1/4 or 1/3 of the labia minora but partial fusion has also been observed anteriorly in the vulva. In the present material, complete and partial adhesions occurred equally frequently with 6 cases in each group while only 2 cases of the type with multiple perforations occurred.

2 Age at which the condition is observed

Observations in the literature appear to be unanimous (5, 10, 14, 15) that vulval synechia are observed chiefly within the first 2 years of life although the limits were wide (from 2 months to 7 years). This, however, gives no information about the time of origin of the adhesions. The present authors have made the same observation as the adhesions in the 14 patients concerned were noted for the first time between 5 months and 6 years of age in 12 cases within the first 2 years of life. The authors have never observed adhesions in female infants under the age of 5 months.

3 Incidence

It has not proved possible to obtain any concrete figures for the incidence of vulval synechia. The majority of authors state that ad-

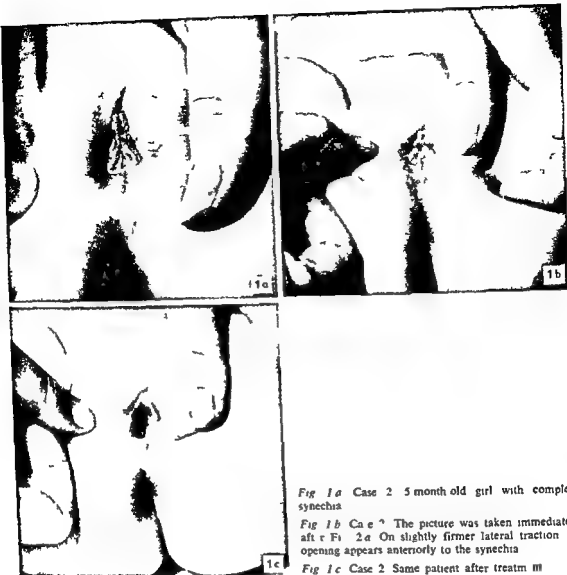


Fig 1 a Case 2 5 month old girl with complete synechia

Fig 1 b Case 2 The picture was taken immediately after Fig 2 a On slightly firmer lateral traction an opening appears anteriorly to the synechia

Fig 1 c Case 2 Same patient after treatment

lesions occur quite frequently Barysh (2) reports that as a paediatrician he sees from 6-12 cases requiring treatment annually and this is in good agreement with Finlay (10) who also reports having seen the same number of cases annually Finally the material reported by Nowlin et al (15) which originates from an urological and a paediatric department comprises 110 cases observed in the course of 14 years This corresponds to the admission of 7-8 children with vulval synechia annually No information is available however concerning the total

Seven out of the 14 patients in this material originate from children hospitalized for other reasons Four of them were found during the year 1969-70 in which all of the girls admitted a total of 287 aged 0-14 years were examined systematically in view of vulval synechia The incidence was thus 1.4% Out of the seven girls referred from general practice 4 were found in 1969 and the other 3 in the first half of 1970 It is strange that no other general practitioner observed any cases They admit however that inspection of the external genitalia is as a rule too superficial



Fig 2a Case 1 5 month old girl with partial synechia located to the lower half of the vulva cleft



Fig 2b Case 1 After treatment

4 Symptoms and complications

As mentioned previously vulval adhesions rarely cause any symptoms. Difficulties with micturition and urinary infections have been described however (2, 13, 18, 19, 21), and Nowlin et al (15) found such symptoms in 20% of 110 girls. None of the 14 patients in the present material had local or urinary symptoms, and urinary infection was not demonstrated in any case.

5 Etiology and pathogenesis

In discussion on the etiology of the condition considerable interest has been focused on the question of the condition being congenital or acquired. As early as 1825, Dewees (8) maintained that the condition is practically always acquired, a conception which is supported by the majority of authors (4, 15). Campbell (5, 6, 7) considers, however, that the condition is congenital but that it is seldom revealed at birth. He considers that the congenital form was present in 39 cases. It is however not immediately apparent from his statement whether a personal observation or an opinion is con-

cerned. The only definite case with congenital vulval synechia was described by Bowles & Childs (4) out of a total of 20 cases observed. The problem is most convincingly illustrated by Finlay (10) who examined all newly born female infants in an English maternity department in view of both vulval synechia and congenital dislocation of the hip. Out of a total of 5 000 newly born female infants he did not encounter any congenital cases of the former condition.

Dewees (8) considered that inflammation of the mucous membrane of the labia resulting from poor hygiene might be the cause of the fusion. There can hardly be any doubt that fusion of the mucous membranes may occur in connection with prolonged inflammatory conditions (25) although it is far from proven that this is as a rule the cause of the condition described here. Some authors consider that slight cases of vulvitis (8, 22, 23) or mechanical irritation (4, 10) possibly in connection with the great cellularity and vascularity in the labia minora (12, 15) may be of significance in the pathogenesis.



Fig 3a Case 7 14 month old girl with an opening both anteriorly and posteriorly



Fig 3b Case 7 After treatment

Anderson (1) refused however to accept this explanation. He maintained that he had never observed any connection between genital hygiene and the occurrence of synechia and added that a possible explanation for the presence and recurrence of these adhesions is that the mucus secretions in such girls contain a certain musilaginous property that causes these two opposing surfaces to adhere. This is pure speculation.

Finally Teton (22) in 1956 mentioned that adhesions of the labia minora via a non specific vaginitis might be "due to an exaggeration of the normal hypoeostrogenism of childhood".

Williams & Cramm (24) agreed with this conception which may also explain why the condition is not observed in newly born infants and is rare in women after the *menarche* (6, 12) as increase in the circulating oestrogen occurs in both of these age groups. The condition has however been described in adults (21). Finally Dewhurst (9) considered that this theory was supported by the observation that

oral administration of oestrogen appears to result in disappearance of the adhesions. This entire question seems to be very debatable and no investigations of oestrogen levels in girls with vulval adhesions are available.

The present authors have never observed synechia in newly born infants and vulvitis could not be demonstrated in any of the 14 cases reported here.

6 Treatment

In his early investigations Dawes (8) pointed out that the condition may disappear spontaneously and this has since been supported by other authors (1, 10). The majority of authors still maintain that no treatment is necessary for small partial adhesions. Complete or extensive partial synechia and cases causing local symptoms or urinary infections should be treated. The majority of authors consider that the membrane should be incised or divided in the mid line (2, 5, 8, 11). As mentioned previously Teton (22) considered that the condi-



Fig. 2 a Case 1 5 month-old girl with partial synechia located to the lower half of the vulva cleft

Fig. 2 b Case 1 After treatment

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Key words Adhesions of labia minora synechia vulvae vulvar fusion agglutination of the minor labia

tion could be treated successfully by oral administration of oestrogen while Williams & Cramm (24) found that local application of oestrogen ointment had an equally good effect.

Treatment such as this may appear drastic and unnecessary for a condition which as in the 14 cases presented here, may be treated without undue discomfort by lateral manual traction of the labia majora which causes the membrane to rupture without haemorrhage.

7 Prognosis

It is apparent not only from the literature (5, 16, 22) but also from the authors' experience that follow up treatment for a week or more with application of an indifferent ointment to prevent the formation of new adhesions between the raw mucosal surfaces, is very important. Nowlin et al (15) found, that despite this procedure one or more recurrences took place in 20% of their 110 patients. In the present series recurrence was observed in two out of 14 patients, i.e., approximately the same proportion (14%) but it was obvious that these 2 cases occurred because the follow up procedure and control were defective. No untoward psychological effects attributable either to the condition or to the treatment have been observed in the children. All of the parents expressed their satisfaction that the treatment was so easy and their relief that the child was now normal.

SUMMARY

The diagnostic and therapeutic problems in 14 cases of adhesions of the labia minora in children are mentioned. It was found that the condition is quite frequent, is acquired and occurs predominantly during the first 2 years of life.

Thorough investigation of the external genitalia in little girls is essential to establish the diagnosis. This is important because the treatment, in which the adhesions are ruptured by means of slight lateral traction, is very easy and painless and because the untreated condition may result in urinary symptoms. Follow up

treatment with an indifferent ointment applied to the exposed epithelial surfaces is important in order to avoid recurrence.

Recognition of the condition is important as incorrect interpretation as pseudohermaphroditism may cause much anxiety and trouble for the children and their families.

The pathogenesis is unknown and the hypothesis that it is due to an exaggerated form of the normal childhood hypoestrogenism in the affected children deserves further investigation.

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Fig 1 The patient aged 16 years and two months.

somes showed a section of bright fluorescence on the distal segment of the long arm (Fig. 4). The analysis of eleven cells from skin cultures showed five of them to have 45 chromosomes (consistent with an XO constitution) and two 46 chromosomes. The analysis of nine cells from cultures of fallopian tube tissue showed in four of them 46 chromosomes and

in five 45. Both in skin and in fallopian tube culture the cells with 46 chromosomes had five achrocentrics as in peripheral blood cultures.

COMMENT

The presence of short stature, mental retardation, widely spaced nipples, skeletal alterations such as hypertrophy of the medial condyle of the tibia and small pituitary fossa, and other clinical minor abnormalities in a subject with sexual hypoplasia and streak gonads suggested the diagnosis of gonadal dysgenesis with many of the features of Turner's syndrome. Blood lymphocytes showed 46 chromosomes with five small achrocentrics. One of these, although a little larger, could not be interpreted as a Y on its morphological appearance alone. The autoradiography was not informative. No hot chromosome was found among 40 labelled metaphases and this finding suggested that the abnormal chromosome was not an X fragment. Identification of this chromosome was achieved by applying fluorescence technique to peripheral blood cultures. We demonstrated a fluorescent section corresponding to the distal segment of the long arm of one of the five achrocentrics; this phenomenon has been recently demonstrated to be characteristic of the Y chromosome (8, 6). A sex chromosome complement of the XY

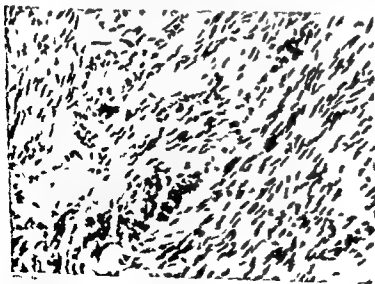


Fig 2 Histology of the biopsy fragment from the right gonad.

CASE REPORT

IDENTIFICATION OF THE Y CHROMOSOME BY THE
FLUORESCENCE TECHNIQUE IN AN XY/X0 GONADAL DYSGENESIS

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We report in this paper a case of gonadal dysgenesis with XY/X0 chromosome sex complement. The Y chromosome was definitely identified with the aid of the fluorescence technique.

CASE REPORT

Gianna R. this Italian girl was born on April 25th 1954 when her mother and father were respectively 24 and 26 years old. She was the product of a normal pregnancy, labor and delivery. The mother did not take any drugs neither was she exposed to radiation during pregnancy. The child's birth weight was 3250 g. She was the first of three siblings. Two sisters are normal. The parents are not consanguineous. They had observed that the patient's growth was stunted from her second year of life and later that she was slightly mentally retarded. At the age of 14 years she weighed 25 kg and was 128 cm in height corresponding to a height age of 8 years and 8 months (1). Signs of sexual development were absent. At the age of 16 years and 2 months (16.6.1970) she was admitted to the Paediatric Clinic of the University of Pavia for investigation of dwarfism and sexual infantilism.

Physical examination showed a patient of definite female phenotype but with a development corresponding to an age of about 10 years (Fig. 1). Weight 27 kg, height 133 cm, height age 9 years and 8 months (1), lower segment 67 cm, upper/lower ratio 0.98, span 139 cm, head circumference 54 cm, chest circumference 68 cm. Hypertelorism and slight arched palate were present. The areolae were large but not low set and the occipital hairline slightly low. The neck was short but not webbed. Breast development was absent and the nipples were widely spaced. Slight

cubitus valgus was present. External genitalia were morphologically normal but underdeveloped. Little sexual hair was present on the labia majora. Heart sounds were normal as were the electrocardiogram and the phonocardiogram. Liver and spleen were not palpable. Fundus oculi was normal. The Wechsler Intelligence Scale score was 61. Skeletal X-ray examination showed a skeletal age of 11-12 years (2). Normal metacarpals, hypertrophy of the medial condyle of the tibia. Parietal and frontal bones were thickened and the pituitary fossa was small. A pyelogram showed a normal urinary tract. A vaginogram showed the existence of a vagina about 4 cm in length. The urinary oestrogens excretion was 2.6 µg/24 hrs and the urinary 17 ketosteroids excretion was 4 mg/24 hrs. The urinary gonadotropins excretion fluctuated between 336 and 800 RMQ IRP IU/24 hrs on twelve consecutive days. Dermoglyphs were normal. Endoscopic examination by Wolff's photocoelioscope showed a very hypoplastic uterus and short and thin Fallopian tubes. Gonads were represented by two club-shaped streaks with a smooth surface. Biopsies were taken from the right gonad (for histological examination), the right tube and the skin (for chromosomal study). On histological examination the right streak gonad consisted of abundant connective tissue compressing residual epithelial cords (Pflüger's cords) and was completely devoid of follicles (Fig. 2). Sex chromatin was absent in a buccal smear. The examination of 46 cells from blood cultures showed in all of them 46 chromosomes with five small acentric chromosomes (Fig. 3).

Autoradiography was performed labelling a blood culture for the last four hours with 0.5 µCi/ml of tritiated thymidine (Amersham s.a. 6 Ci/mM). No obvious late replicating chromosome was found in 40 labelled metaphases analysed. Fluorescence technique was applied to slides from blood cultures stained with quinacrine dihydrochloride according to Pearson et al. (6). One of the five small acentric chromo-

whereas ovarian follicles have been clearly demonstrated only in the case reported by Hirschhorn (4, 5). This finding agrees with the hypothesis that an XY/XO mosaic arises from the loss of a Y chromosome during an early mitotic division of an XY zygote. Thus far it is not possible to establish if the variety of clinical pictures and of gonadal alterations is related to the relative proportion of cells with 45 and with 46 chromosomes in various tissues. Finally as far as our case is concerned the clinical features and the histological findings are in agreement with a diagnosis of Turner's syndrome: the karyotype was established as an XY/XO mosaic in which the Y chromosome has been identified by the fluorescence technique. This technique allows an easy identification of the Y chromosome in cases like the present one in which a differentiation between the Y chromosome and X fragments is needed.

SUMMARY

A phenotypic female aged 16 years had sexual hypoplasia, streak gonads and sex chromosome mosaicism. She was XY in peripheral blood cultures and XY/XO in Fallopian tube tissue and in skin cultures. A definite identification of the XY line was made by the fluorescence technique which showed a fluorescent section corresponding to the distal segment of the long arm of one of the five acrocentrics.

ACKNOWLEDGEMENT

We are indebted to Dr A. Sallusto of the Obstetric Clinic of the University of Pavia for performance of the coelioscopy. This work has been aided in part by a grant from CNR, Rome.

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Key words: Gonadal dysgenesis, mosaicism, Y chromosome identification, chromosome fluorescence.

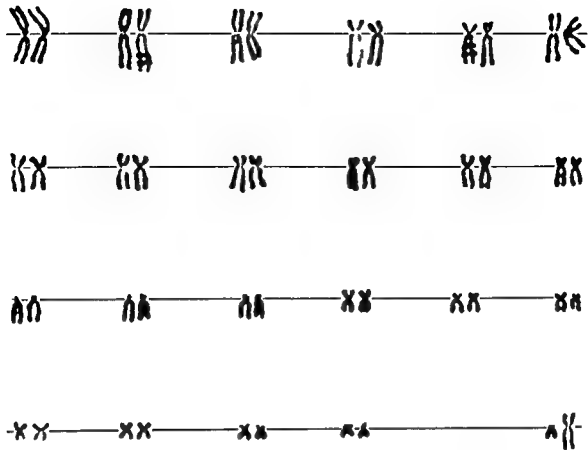


Fig 3 Karyotype of a cell from peripheral blood culture with 46 chromosomes

type in a female phenotype has been usually found in pure gonadal dysgenesis and only in very few patients with the features of Turner syndrome (3). We therefore suspected the existence of a tissue mosaic which was confirmed by the chromosomal analysis of the Fallopian tube tissue and of the skin which were found to be XY/XO.

This type of mosaicism corresponds to a large variety of gonadal abnormalities and of clinical pictures. It was reported chiefly in mixed gonadal dysgenesis but also in male pseudohermaphroditism in the so called 'male Turner syndrome' and in few cases of Turner's syndrome. According to Van Campenhout (7) out of 51 cases with XY/XO sex chromosome complement and with bilateral gonadal identification 38 had testicular tissue,

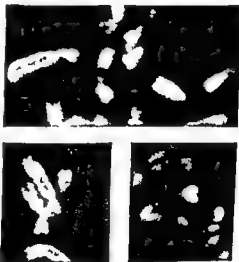


Fig 4 Details of three cells from peripheral blood culture stained with quinacrine dihydrochloride showing a fluorescent section on the distal portion of a small acrocentric chromosome

whereas ovarian follicles have been clearly demonstrated only in the case reported by Hirschhorn (4, 5). This finding agrees with the hypothesis that an XY/XO mosaic arises from the loss of a Y chromosome during an early mitotic division of an XY zygote. Thus far, it is not possible to establish if the variety of clinical pictures and of gonadal alterations is related to the relative proportion of cells with 45 and with 46 chromosomes in various tissues. Finally as far as our case is concerned the clinical features and the histological findings are in agreement with a diagnosis of Turner's syndrome: the karyotype was established as an XY/XO mosaic in which the Y chromosome has been identified by the fluorescence technique. This technique allows an easy identification of the Y chromosome in cases like the present one in which a differentiation between the Y chromosome and X fragments is needed.

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CASE REPORT

CONGENITAL LEUKAEMIA

J HAAHR and A B HALVEG

From the Paediatric Department and the Pathological Institute Copenhagen County Hospital Glostrup Denmark

Leukaemia in the newborn infant is extremely rare. The clinical picture in this age group deviates on several points from that of leukaemia developing later in life. The disease is manifest even during foetal life and, consequently, several interesting aetiological aspects are associated with this disease. In this paper one case of congenital leukaemia will be discussed on the basis of these considerations.

CASE REPORT

In april 1969 a newborn boy was transferred from the maternity ward to the paediatric department of the Glostrup Hospital because of wide spread haemorrhages in the skin. The mother was 20 years old, she was pregnant for the second time and had two years earlier given birth to a normal girl. The mother had always been healthy and was still so at the follow up in July 1970. There was no family history of malignant diseases. Pregnancy had been uncomplicated without exposition of the mother to roentgen examination, drug treatment or infectious diseases. Delivery after puncture of the membrane was normal. Birth weight 3 140 g, length 52 cm. The placenta was normal. Apgar score 3 after one minute and 7 after 10 min.

Minor cutaneous infiltrations reminding of tumour dissemination were seen immediately at birth as well as haemorrhages in the skin (Fig 1). There was moderate swelling of the liver and spleen and the inguinal lymph nodes were the size of beans. The general condition of the infant was surprisingly good.

Some of the results obtained by examination of peripheral blood and bone marrow are recorded in

Table 1. The blood showed marked leucocytosis with many promyelocytes, myelocytes and stem cells (Fig 2).

Other examinations. The bleeding time exceeded 10 min. Blood urea 0.42 g/l. GP transaminase, alkaline phosphatase, serum protein, electrophoresis and immunoelectrophoresis of serum, normal findings. The blood contained no thrombocyte antibodies and the titre of cytomegalovirus antibodies was normal. The parotitis complement binding reaction was negative. Culture from the bone marrow showed growth of staphylococcus albus (probably contamination), but culture from blood and spinal fluid was negative. Urine microscopy was normal without owl eye cells. ECG normal. Roentgenograms of the chest, long tubular bones, the skull, the pelvis and the spine were normal. Ophthalmoscopy was normal. Chromosome investigation was attempted on peripheral blood and bone marrow from mother and child but without success.

In the maternal blood the following were normal: Hb, leucocyte and thrombocyte counts, serum electrophoresis, cytomegalovirus antibody titre, parotitis complement binding reaction, Wassermann reaction. Biopsy of the bone marrow showed nothing abnormal. Alkaline phosphatase 8 (decreased). No owl eye cells in the urine. IgA and IgM in the serum were lowered and haptoglobin was increased.

Despite treatment with parenteral fluid, hydrocortisone and vincristine the patient died on the 7th day of life.

Autopsy. No malformations were found. Leukaemic infiltrations were seen in the lymph nodes, bone marrow, lungs, liver, spleen, kidneys and pancreas (Fig 3).

DISCUSSION

de Pollmann (15) in 1898 described the first case about 50 cases have been reported,



Fig 1 Cutaneous infiltrations in a new born boy with congenital leukaemia

Table 1 Results of differential count in blood and bone marrow

	1st day of life		4th day of life	
	Marrow	Blood	Marrow	Blood
Hb g/l		116		138
Thrombocyte count / l		79 000		67 000
Leucocyte count / l		311 000		352 000
Hemocytoblasts (Stem cells)	76	25	76	70
Pr myelocytes	43	38	2	4
Myelocytes	11	18	1	1
Metamyelocytes	6	7	3	4
Neutrophil granulocytes	1	7	12	15
Lymphocytes	2	3	3	2
Basophil erythroblasts	9	—	—	—
Polychromatophil erythroblasts	1	1	—	—
Normoblasts	1	1	3	2
Total count	100	100	100	100

but some of these appear doubtful. The cases have occurred without any geographic accumulation except that a rather high number

have been reported from Scandinavia (2, 6, 9–12, 18, 19). It has been attempted to classify congenital leukaemia into various types according to the time of onset of symptoms (4). According to Dalgaard & Kaas (6) the disease should only be considered congenital if the symptoms or clinical findings are manifest immediately at birth or within the first week of life.

The diagnosis is not correct unless immature leucocytes and stem cells are present not only in the ordinary extramedullary haemopoietic organs but also in non haemopoietic organs (4, 14, 17, 18).

Congenital leukaemia is generally of the myeloid type (80%) as a rule it is associated with marked leucocytosis in which promyelocytes predominate both in the peripheral blood and in the bone marrow. Some cases however must be classified as stem-cell leukaemia. Anaemia which rarely is manifest at birth de-

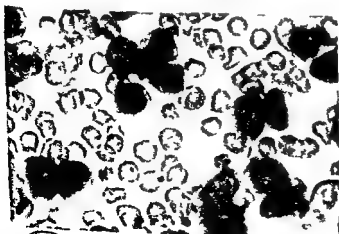


Fig 2 Peripheral blood

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DISCUSSION

Since Pollmann (15) in 1898 described the first case about 50 cases have been reported.

influenced by one or several genes localized in chromosome 21. Except for these studies the occurrence of leukaemia in newborn infants does not give any clue to the aetiology.

SUMMARY

A report is submitted of a case of leukaemia of myeloid type in a newborn boy who died on the 7th day of life, despite anti leukaemic therapy. The available reports on congenital leukaemia and on children born of mothers with leukaemia in pregnancy do not give any clue to the aetiology.

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Fig. 3 Leukaemic infiltrations in the pancreas

velops shortly after, while thrombocytopenia usually is found from the beginning. Cutaneous and mucosal haemorrhages, together with swelling of the liver and spleen, are common clinical findings, whereas lymph node swelling is rare. Nodular skin infiltrations is a characteristic finding.

The differential diagnoses include leukaemoid reactions attributable to sepsis, congenital syphilis, erythroblastosis and congenital thrombocytopenic purpura.

Treatment has so far been without effect and generally death occurs within few days (4), although a few cases surviving several months have been observed; the latter are seen mainly among patients with Down's syndrome (7, 8, 13).

The aetiology of congenital leukaemia is just as obscure as that of other types of leukaemia. The case histories hitherto reported fail to provide any data concerning factors commonly considered responsible (14). Among the mothers to children with congenital leukaemia none had leukaemia during pregnancy. One case is on record, however, in which leukaemia was diagnosed in the mother immediately after parturition, and acute lymphatic leukaemia developed nine months later in the infant (5). Generally, congenital leukaemia must be induced by factors which do not affect the mothers. An intra uterine factor seems

to inhibit the leukaemic process in the fetus, since the symptoms often are slight immediately after birth but aggravate rapidly afterwards (14). If a virus were assumed to be involved, it must act upon the child without affecting the mother but apparently this does not agree with the well known fact that mothers affected with leukaemia during pregnancy give birth to healthy children (3). If hereditary factors were involved, an increased incidence of leukaemia among children born of leukaemic mothers might be expected, but this is apparently not the case (1).

It is a well-documented fact that the incidence of leukaemia in patients with Down's syndrome is higher than that in the general population. The same applies to congenital leukaemia: in a series of 45 cases, 10 were found in Down's syndrome (13). It is of interest that patients with chronic myeloid leukaemia usually present with a chromosome anomaly including deletion probably from a chromosome in the G 21 group, the so called Philadelphia chromosome. In these patients, the alkaline phosphatase level in leucocytes is rather low in contrast to the findings in patients with Down's syndrome of the trisomy-21 type in whom the level of alkaline phosphatase in leucocytes is elevated (4, 16, 20). Even though this is not fully elucidated, chromosome studies so far suggest that the leucopoiesis is

influenced by one or several genes localized in chromosome 21. Except for these studies the occurrence of leukaemia in newborn infants does not give any clue to the aetiology.

SUMMARY

A report is submitted of a case of leukaemia of myeloid type in a newborn boy who died on the 7th day of life despite anti-leukaemic therapy. The available reports on congenital leukaemia and on children born of mothers with leukaemia in pregnancy do not give any clue to the aetiology.

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Key words: Congenital leukaemia



Fig 3 Leukaemic infiltrations in the pancreas

velops shortly after birth while thrombocytopenia usually is found from the beginning. Cutaneous and mucosal haemorrhages, together with swelling of the liver and spleen, are common clinical findings, whereas lymph node swelling is rare. Nodular skin infiltrations is a characteristic finding.

The differential diagnoses include leukaemoid reactions attributable to sepsis, congenital syphilis, erythroblastosis and congenital thrombocytopenic purpura.

Treatment has so far been without effect and generally death occurs within few days (4) although a few cases surviving several months have been observed, the latter are seen mainly among patients with Down's syndrome (7, 8, 13).

The aetiology of congenital leukaemia is just as obscure as that of other types of leukaemia. The case histories hitherto reported fail to provide any data concerning factors commonly considered responsible (14). Among the mothers to children with congenital leukaemia none had leukaemia during pregnancy. One case is on record, however, in which leukaemia was diagnosed in the mother immediately after parturition, and acute lymphatic leukaemia developed nine months later in the infant (5). Generally, congenital leukaemia must be induced by factors which do not affect the mothers. An intra uterine factor seems

to inhibit the leukaemic process in the fetus, since the symptoms often are slight immediately after birth, but aggravate rapidly afterwards (14). If a virus were assumed to be involved it must act upon the child without affecting the mother, but apparently this does not agree with the well known fact that mothers affected with leukaemia during pregnancy give birth to healthy children (3). If hereditary factors were involved, an increased incidence of leukaemia among children born of leukaemic mothers might be expected, but this is apparently not the case (1).

It is a well documented fact that the incidence of leukaemia in patients with Down's syndrome is higher than that in the general population. The same applies to congenital leukaemia in a series of 45 cases 10 were found in Down's syndrome (13). It is of interest that patients with chronic myeloid leukaemia usually present with a chromosome anomaly including deletion probably from a chromosome in the G-21 group the so called Philadelphia chromosome. In these patients, the alkaline phosphatase level in leucocytes is rather low in contrast to the findings in patients with Down's syndrome of the trisomy-21 type in whom the level of alkaline phosphatase in leucocytes is elevated (4, 16, 20). Even though this is not fully elucidated chromosome studies so far suggest that the leucopoiesis is

where children also often complain of stomach pains

On the other hand the gastrointestinal symptoms have whatever their origin an emotional effect on the child and on his parents

Psychotherapy of children with repetitious stomach pains requires a very close collaborative treatment of the mother often with the same therapist

O Koskimies

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting April 3, 1971

K Launiala & J K Visakorpi *Recurrent abdominal pain in children. A review of somatic aspects*

Recurrent abdominal pain (RAP) is well known to be common in children of the age of 5-15 the prevalence being an average of 10-12%. A history of abdominal complaints in the family and symptoms such as sleep disorders, nausea, headache, dizziness, pallor and cold and sweaty hands seem to be common in children with RAP. In clinical examination an organic disorder is found in only 4-8% of children with RAP, the main disorders being urogenital malformations and infections, duodenal ulcer and gallstones in pubescent girls. In these cases the type and localization of the pain is often different from typical transient umbilical pain in children without organic disorders. Recently acquired lactose malabsorption has been shown to be present in many populations of school age children (the prevalence being for example 6% in Finland) and to be the main cause of abdominal complaints (diarrhoea, meteorismus, pain) in milk drinking children with this disorder.

In the diagnosis and treatment of children with RAP excluding organic diseases is most important. With careful history and with clinical examination which includes routine laboratory tests it is often possible to exclude anaemia, occult bleeding in the intestine and urinary abnormalities. X-ray examinations should be performed only if the type and localization of the pain indicate some organic disorder, intravenous pyelography then often being most informative. To exclude lactose

malabsorption a per oral lactose tolerance test is suggested.

Maija-Liisa Koski *Recurrent abdominal pain in children. Psychiatric aspects*

Somatic responses influenced by emotions can roughly be divided into two categories: temporary dysfunctions related to short lived emotions and more lasting functional and structural changes related to long standing emotional conflicts.

Earlier theoretical concepts of the interrelationships between emotions and diseases centered around two specificity theories: Dunbar's theory of specific personality and Alexander's theory of specific conflict. Later the trend has been away from the specificity theories towards more general stress theories.

Gastrointestinal disturbances have been the centre of psychosomatic interest. In childhood, repetitive stomach pains are seen in many different conditions. Colic is one of the earliest gastrointestinal disturbances. The role of constitutional factors has been stressed in producing colic. On the other hand, psychological factors are to be found. It is more common in the eldest child of the family and is almost never seen in hospital. Therefore, it is thought to be related to the mother's tension. Aerophagia is seen in somewhat older children and can persist until adulthood. Anorexia nervosa, peptic ulcer, mucous and ulcerative colitis are among the conditions in which repetitive gastrointestinal pains occur. Frustrated dependency needs have been viewed as the key problem in these conditions as well as in school phobia.

tetracycline and erythromycin. All of the children were over the age of 2 years. With one exception, good agreement was present between the radiological and the clinical and stethoscopic findings.

This work is part of investigations published on a previous occasion on the incidence of m.p. infections among patients admitted to hospital with respiratory infections.

At present insufficient facilities are available for routine investigation for m.p. antibodies in Denmark. It is recommended that all cases of protracted pneumonia with positive cold agglutinin titres be suspected to be caused by m.p. when the common causes of infection have been excluded. This cause is particularly possible when the patients have in addition increase in the streptococcus MG antibody titre as it appears that an antigen relationship exists between the two organisms.

Discussion

Carl Friderichsen Unproductive cough is very characteristic in m.p. pulmonary infections.

Jørgen Kringelbach The stethoscopic findings in primary atypical pneumonias are more pronounced than the radiological findings.

Erik Thamdrup Was culture for virus undertaken?

Jørn Møller No.

Erik Wamberg Were any characteristic pulse changes present?

Jørn Møller No.

Jørgen Bent Andersen The effect of diuretics administered during the latter part of pregnancy on newly born infants.

Alterations in the water and salt relationships were investigated in a number of newly born infants the mothers of whom had been treated with one of the two diuretics Chlorthalidone (Hygroton®) and Bendroflumethazide (Cen-tyl®) during the latter part of pregnancy and until delivery. Indications for the diuretic therapy were tendency to oedema and/or abnormal increase in weight but cases of pre-eclampsia and eclampsia were excluded from the material.

In the infants the following were investigated 2-4 hours after delivery: total body fluid, extra-cellular volume, serum sodium, serum potassium, serum chloride and total osmolality. These values were compared with the values in a group of newly born infants whose mothers had not received diuretic therapy. All six parameters had undergone changes compatible with a diuretic effect on the foetus. The total fluid, extra-cellular volume, serum sodium, serum chloride and osmolality were lowered while the serum potassium had increased. The alterations were slight but the investigation demonstrated that diuretics administered to pregnant women exert a diuretic effect on the foetus also.

Discussion

N. J. Brandt Is the physiological loss of weight identical in infants of mothers treated with diuretics and infants of mothers who had not received this form of treatment?

Bent Andersen The material is too limited to assess this.

Meeting Nov 11 1970

Th. Rosendal Radiographic changes in acyanotic heart disease in infants.

Determination of the volume of the heart/m² body surface by means of the formula for the

volume of an ellipsoid $\left(r = \frac{4}{3} \pi \frac{1}{2} \frac{b}{2} \frac{d}{2}\right)$ on the

basis of the longitudinal, transverse and vertical diameters as measured by X-ray photographs of the thorax in the sitting posture and following correction for enlargement showed in 559 infants with verified congenital heart disease of 31 different types increase in vol-

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE DANISH PAEDIATRIC SOCIETY

Meeting Sept 9, 1970

Kristine Hauge Kristensen, Knud E Petersen & Erik Thamdrup *Acute suprarenal cortical insufficiency in infancy. A case of congenital isolated hypaldosteronism*

A male infant aged 3 weeks was admitted on account of failure to thrive. Pregnancy and delivery had been normal. On admission the weight was 230 g below the birth weight and the infant was dehydrated with hyperkalaemia and hyponatraemia. A brother and an uncle had died in infancy with similar symptoms.

Following a review of the differential diagnostic possibilities in cases of salt loss in infancy it was considered that the cause was not to be found in the kidneys although the child had pyuria and vesico-ureteric reflux. Congenital hyperplasia of the suprarenal cortex (adrenogenital syndrome) with salt loss could be excluded on account of the normal excretion of 17 ketosteroids and the absence of pregnantriol in the urine. Congenital hypoplasia of the suprarenals was another possibility. The clinical picture corresponded exactly to the symptoms exhibited by the chil-

dren with isolated hypoadosteronism described by Visser, *inter alia*. Investigations were therefore rapidly focused on this.

Normal cortisol production (secretion rate) with response to ACTH was encountered while the aldosterone production was practically nonexistent and did not respond to pronounced salt loss despite adequate stimulation via the angiotensin-renin system. Corticosterone production was greatly increased as evidence of a block in biosynthesis between corticosterone and aldosterone. Future investigations will reveal whether a defect in 18-hydroxylase or in 18-hydroxy dehydrogenase is concerned.

Treatment of these patients consists of administration of sodium chloride supplements and desoxy corticosterone. It is emphasized however that there is a tendency for the salt loss to diminish during the first years of life so that treatment should be followed up and possibly stepped down. Apart from the above mentioned symptoms, the infant appeared to have brain damage with retarded psychomotor development.

Meeting Oct 14 1970

Jørn Møller *Infections with mycoplasma pneumoniae in children*

A review of the infectious qualities of mycoplasma pneumoniae (m.p.) is followed by a report of 5 cases of pneumonia probably caused by this organism. Isolation of the organism in cultures from throat swabs and four fold in-

crease in the titre of antibodies to m.p. are greatly suggestive of these infections. It is emphasized that m.p. grows very slowly even under optimum conditions *in vitro*. In all of the cases increase in the titres of cold agglutinins were demonstrated. The infections ran protracted courses and appeared to react to

teracycline and erythromycin. All of the children were over the age of 2 years. With one exception, good agreement was present between the radiological and the clinical and stethoscopic findings.

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Determination of the volume of the heart/body surface by means of the formula for the volume of an ellipsoid $\left(r - \frac{4}{3} \pi \frac{1}{2} \frac{b}{2} \frac{d}{2} \right)$ on the

basis of the longitudinal, transverse and vertical diameters as measured by X-ray photographs of the thorax in the sitting posture and following correction for enlargement showed in 559 infants with verified congenital heart disease of 31 different types increase in vol-

ume of over 300 ml/m" (normal values for infants) in 75 per cent and up to the double in one third of the cases while the cardio thoracic index was only found to be increased to more than 0.65 (normal value) in 20 per cent. Determination of volume thus permits more accurate determination of whether the heart is pathologically enlarged than measurements of the cardiac ratio and it is of diagnostic value.

The greatest increases in volume are encountered in fibro elastosis and coarctation of the aorta and in cases of large left-right shunt (VSD, PDA and ASD). Increase in volume is however, also found in cases of tumour of the pericardium with fluid accumulation and in tumour of the myocardium and subsequently in glycogenosis, diaphragmatic hernia through the tendineum centrum with elevation of a heart of normal size and in cases of tumour of the thymus.

Determinations of heart volume do not contribute to the differential diagnosis in 361 acyanotic cases of congenital heart disease of 21 different types in infants but may serve to differentiate between the slighter and more severe degrees of the disease.

Where the same group of infants is concerned, the four chambers of the heart, the aorta and the pulmonary artery with its branches in the lungs were assessed on the basis of X ray photographs without knowledge of the verified diagnosis. Uncertainty in the assessment appears from the observation that in 120 infants with normal heart volume (less than 300 ml/m²) enlargement of one or more chambers was registered in 42 per cent and these were registered as normal in 23 per cent of 353 infants with increased heart volume. The reason for this is to be found in the indefinite criteria, the elasticity of the heart and the fact that a combination of different malformations of the heart are frequently present simultaneously.

In a series of cardiac diseases in infants in the acyanotic group and particularly in the severe degrees enlargement of a single cham-

ber or combination of several chambers may produce a characteristic radiographic picture. For example considerable enlargement of the left ventricle combined in a number of cases, with enlargement of the left atrium in fibro elastosis of the left ventricle and atrium and coarctation of the aorta, increase in width superiorly and to the left is observed in corrected transposition, constriction of the pulmonary arch in pulmonary stenosis, prominence of the right border of the heart in ASD and VSD and enlargement of the right ventricle with elevation of the heart in VSD and PDA. Finally, in cases with aberrant pulmonary veins with enlargement of the right atrium and the right ventricle and large superior vena cava and possibly persisting left superior vena cava along or within the upper border of the heart can produce a shadow resembling a snowman.

Variations in the shape of the heart are however so great that alterations in the shape of the heart only provide limited assistance in the differential diagnosis. In the largest group of 83 infants with verified VSD with pressure in the right ventricle of greater than 40 mmHg and increased heart volume to more than 300 ml/m² practically every possible combination of dilatation of the various chambers of the heart was encountered.

Alterations in the vascular shadows in the lungs are of much greater diagnostic significance. Reduced vascular markings were shown only in cases of pulmonary stenosis in this series. Increased vascular markings were encountered in up to 50 per cent of cases with left-right shunt without alterations of pressure in the right ventricle and up to 89 per cent when the pressure in the right ventricle was increased to more than 40 mm Hg, so that this is an important criterion in left-right shunt although it does not render any definite information concerning the localization of the shunt. Simultaneously prominence of the pulmonary artery arch suggests ASD or PDA but occurs only in half of the patients with congenital heart disease of these two types.

Transition of left-right shunt to right-left

shunt in connection with progression of pulmonary hypertension may become apparent in ordinary X rays as diminution of vascular markings and increasing prominence of the pulmonary arch

Discussion

Bent Friis Hansen How many infants without heart disease have nevertheless enlarged hearts? In practice it is a difficult problem to decide whether an infant with cyanosis or respiratory distress has an organic heart disease or an enlarged heart merely on account of anoxia or anaemia. Further it would be interesting to know how many completely healthy children have enlarged heart volumes.

Th. Rosendal I have no experience of children in the first year of life.

J. Vesterdal Has the phase of respiration been taken into consideration? When the infant cries great changes can occur in the size of the heart and the degree of filling of the pulmonary vessels. When the child presses the heart becomes smaller and the pulmonary vessels empty and the reverse occurs during forced inspiration. How can it be ascertained whether the alterations observed in Dr Rosendal's material are genuine?

Bent Friis Hansen Does the position of the patient (lying or sitting) play any part for the determination of heart volume?

Th. Rosendal (to J. Vesterdal and Bent Friis Hansen) I do not think that heart volume determinations are influenced by the position of the patient. Nor do I consider that the phase of respiration plays any part.

lb. Boesen The X ray picture of a lying patient is distorted and cannot be employed for determination of volume. The phase of respiration is of importance and the picture should be taken in inspiration.

S. Brandt & H. Brammer Demonstration of four patients with good recovery from infantile spasms and hypersarhythmia.

A brief introductory review was given of 18 patients treated with steroids in Queen Louise's Hospital for Children during the period 1959-69.

Eight of these patients had other serious neurological conditions simultaneously. Two of the patients had oligophrenic siblings. All of these 10 became mentally retarded.

In the remaining 8 patients no known pre-disposition to neurological conditions was encountered and no neurological conditions other than infantile spasms were present.

Four of these patients became mentally retarded although subsequent follow up showed distinct mental progress as assessed by intelligence tests.

The remaining patients developed normally both mentally and motorically. These patients were demonstrated. Three of them had shown psychomotor retardation on admission. One of these had gradually regained normal mental status. At subsequent tests the IQ was found to increase until it became normal 5 years later.

In the remaining patients normal findings were encountered after cessation of steroid treatment.

Discussion

J. Vesterdal Have there been any new findings concerning the etiology and pathological anatomy? In particular can anything be said about the relationship to whooping cough vaccination?

J. C. Melchior In reply to the question about the connection between infantile spasms and vaccination reference is made to the work in *Ugeskrift for Læger* 131 746-748 1969.

I consider that the vast majority of cases are due to temporal coincidence and a similar opinion was expressed at a meeting of European Child Neurologists in Oxford in September 1970 and supported by participants from 16 countries.

Conversely it appears to be impossible to deny that in isolated cases vaccination may be

a cause when it is considered that any form of injurious influence on the CNS at a certain stage of the development may result in the symptom of infantile spasms

As a reply to J. Vesterdal who sought information on the etiology and the pathological anatomy, I can refer to the work undertaken by Erna Christensen and myself (*Danish Medical Bulletin* 7:121-127, 1960) in which it was demonstrated that practically every possible form of pathological change may be anticipated in the central nervous system and that the decisive factor is probably a discrepancy between the development of the CNS and the chronological age of the child

The results of these investigations have since been confirmed from numerous other sources and are supported also by subsequent reports of materials from Rigshospitalet

Erik Thomsen We have now adopted early vaccination for whooping cough. Has this altered the incidence of infantile spasms?

J. C. Melchior We dare not make any statement about changes in the incidence but we have observed infantile spasms in connection with vaccination for diphtheria and tetanus, i.e. about the age of 5-9 months

B. Brammer It is possible that the prognosis is better in patients in whom no other neurological conditions can be demonstrated and who are not predisposed to neurological conditions

Jørgen Haahr Malformations of the urinary tract in children with urinary infections

During the period I VII 1964 to 31 XII 1968, a total of 95 children were admitted to Queen Louise's Hospital for Children with verified urinary infections. By urinary infection is understood here growth of at least 100 000 bacteria/ml in a mid stream specimen of urine and leukocyturia with at least 5 leucocytes per field in the centrifuged urine. Malformations were encountered in 68 per cent. Vesico ureteric reflux and obstruction of the urinary passages were the most frequent malformations

Twenty eight out of the 65 children with malformations had two or more malformations of the urinary tracts

Obstructive lesions of the urethra were suspected in 13 children but verified in 9 cases only. Three children had urethral valves, two sclerosis of the sphincter, two organic meatal stenosis, one double urethra and one a urethral stricture. It is emphasized that more attention should be focused on the lower urinary passages. With the possible exception of simple dilatation of the urethra, surgical intervention should, however, be delayed until the obstructive urethral lesion is verified and this should be undertaken on the basis of the total assessment of the results of the various investigations as all of these reveal great physiological variations in normal children

Jørgen Haahr Vesico-ureteric reflux in children

In a material from Queen Louise's Hospital for Children consisting of 95 children with urinary infections vesico ureteric reflux was demonstrated in 38 (40 per cent). 25 girls and 13 boys. All of these were followed up regularly in the out patient department for a period of observation of approximately 3 years. Twenty three of the patients had reflux alone and the other 15 had additional malformations of the urinary tracts including urethral obstruction in 8 cases and double anlage in 5 patients

Children with uncomplicated reflux, i.e. without other malformations of the urinary tracts had as a rule a maximum of two episodes of urinary infection while children with complicated reflux had five or more episodes of urinary infection during the period of observation

The 38 children sustained a total of 15 urinary infections of which less than half were asymptomatic. Pyrexia was the most common symptom in the remaining cases

Only 26 children were followed up for a minimum of 1 year. In 12 children treated cor-

sensitively the reflux had ceased in four. In 14 children a total of 18 ureters were implanted and the reflux was successfully relieved. In six of these remained unchanged in 9 cases and in 3 cases no investigation has been performed since the last operation. The reflux disappeared in 3 children only in the group submitted to operation in one case following implantation and in two following dilatation of the urethra for organic meatal stenosis.

Discussion

Jørgen Vesterdal: Dr Haahr's figures demonstrate how few of the children complain of dysuria and that pollakisuria was observed in only a few. Although these symptoms are not observed in the majority of cases simply because the children are so small the investigation reveals nevertheless that investigation of the urine is essential in order to exclude infection. Urinary infections are frequently symptomatic.

I. Hansted: Is the treatment different for massive and for minimal reflux?

Haahr: No differentiation was made in this material.

C. Højensgard (guest): Cystoscopy was undertaken only in a minority of the patients in the material presented. This investigation is an important and necessary part of the urological investigation of children with recurrent urinary infections particularly in view of the indications for operation in cases of vesico-ureteric reflux. I do not consider that the investigation involves an undue risk of infection or trauma when compared with the serious nature of the underlying condition.

Cystoscopy renders primarily important information concerning the appearance and function of the ureteric ostia. In severe cases of reflux large permanently gaping ostia are encountered. There is definite difference between this form of malformation of the ostium and the ostium which is gaping on account of infective oedema of the mucosa. Frequently

diverticulum of the bladder wall (sacculus) is observed at the site of the junction with the ureter suggesting insufficient mural support at the lower part of the ureter. It is obviously important to note the location and number of the ureteric ostia. Valuable information may be obtained by observation of the ureteric ostia during micturition which is an entirely different process from their behaviour during passive distension of the bladder. Employment of the abdominal musculature or pressure over the symphysis. In many cases it is possible to obtain micturition around the cystoscope when the patient is waking up following suitable anaesthesia. Finally cystoscopy renders information concerning other important conditions primarily signs of infection (particularly in cystitis cystica) and trabecular markings.

At present uncertainty reigns as to the extent to which children with recurrent urinary infections should be submitted to operation and which technique should be employed. In holding we have a group of patients in whom we consider operation is absolutely essential on the basis of the clinical picture (failure of medical treatment), radiographic findings (massive reflux with dilatation) and cystoscopy (ostia which are pathological in appearance and function). I shall not attempt to consider the operative methods here.

N. Hobolth: As Højensgard has described we feel convinced that a differentiation must be made between slight and massive reflux. In the latter filling of the entire ureter and the pelvis is observed in the micturition cystography and with practically no exceptions cystoscopy reveals either golf hole or stall door ostia. In our material of 34 patients there were 14 cases of reflux 11 of which showed massive reflux in one or more ureters. With the exception of a single patient who had very severe primary symptoms we waited for one or more recurrences to take place before undertaking operation. All of the patients however were submitted to reflux inhibiting operation. Numerous patients could be dis-

a cause when it is considered that any form of injurious influence on the CNS at a certain stage of the development may result in the symptom of infantile spasms

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BOOK REVIEWS

Ingrid Gamstorp *Pediatric neurology* Appleton Century-Crofts New York 1970 394 pp illus 180s

Pediatric neurology is today a rapidly expanding field requiring increasingly specialized knowledge not least in diagnostic developmental medicine. There is a great need for comprehensive and clinically oriented up-to-date informational material for post graduate education. Ingrid Gamstorp, a well-known Swedish pediatric neurologist with a vast and broad based clinical experience, has taken upon herself the large task of writing a book surveying the main topics that have to be known by everyone working with children suffering from neurological disorders or developmental delay. Dr Gamstorp has chosen the very attractive and practically oriented approach of dividing her book into chapters according to the nature of the symptoms. In addition to self-evident headings such as convulsions, cerebral palsy, mental retardation, headache etc., there are also interesting differential diagnostic titles such as "Generalized muscular hypotonia and flaccid weakness", "Abnormal muscle stiffness", "Localized weakness".

Ataxia, Involuntary movements etc. Thus the book is really written for everyday practice in wards and out-patient clinics. In general, the book is clearly written and gives adequate and up-to-date information. It has well selected and modern references, a good reference system between the various chapters and is extremely well illustrated. This applies particularly to the chapter covering the neurologic and special examination and those treating neuromuscular weakness. The section about mental retardation is less well balanced between common and very uncommon causes and lacks a didactic classification.

In the introduction one seeks in vain for information as to what type of doctor this book has been directed to. To me it seems clear that this is the ideal textbook for the general pediatrician and the pediatrician under training who wants to learn more about the examination methods and the syndromes involved in pediatric neurology. The same is also valid for the doctor with general neurological experience working with children. In addition, it is a good introductory source of information for those who really want to subspecialize in pediatric neurology. However, the compactness of the book (384 printed pages) tells us that the aim is not to be a substitute for complete and extensive specialized textbooks, of which I think this work is the class in the

It would be surprising if the reviewer agreed with the author on all points. Dr Gamstorp has a tendency to be somewhat categorical in some of her statements, even in recognizedly difficult and controversial fields of management where more than one approach or opinion might be correct. This applies for example to the treatment of subdural hygroma (p. 224) where only the burr hole method is accepted, subdural shunts not even being mentioned. Her fears concerning diagnostic and therapeutic punctures seem rather exaggerated. Further, it is highly debatable whether an examination of the cerebrospinal fluid must (reviewer's italics) be performed at least once in all children with findings suggesting cerebral palsy, the opposite view being most generally accepted among neuropediatricians. It is furthermore not only cerebellar forms of ataxia (p. 272) that are seen in cerebral palsy. The CSF protein is not high in all types of leucodystrophy, most of the sudanophilic cases having quite normal levels but an abnormal electrophoretic pattern. The categorical and absolute rejection of phenobarbitone for use in older children with grand mal epilepsy is not correct in my opinion, as a not insignificant number of such cases do very well for years on a single nightly dose of 50 mg of this drug. Phenitoin or carbamazepin is not well tolerated, not ideal in all cases.

Most of the figures given are accurate and based on good references. One minor objection! The incidence of hydrocephalus (p. 224) is 2-2.5 per 1000 living newborns only in certain parts of the world, owing to the large geographical variations in the incidence of spina bifida cystica. Figures do exist for the incidence of perinatal and postnatally dependent simple hydrocephalus.

The criticisms made above are however of minor importance. In general, this is a very useful and ambitious book, which I think will help many pediatricians to a better understanding of a field which demands extensive and long clinical experience. Dr Gamstorp has written a monograph with an exceptionally good clinical approach and with the ambition of trying to give her best advice for understanding and helping the suffering child and its parents in all situations. She has indeed succeeded and this work should find its way to the bookshelves of all pediatric and neurologic libraries.

Bengt Håberg

charged from treatment but the period of observation is not sufficiently long for any opinion to be expressed concerning the final re-

sult The operations were, however, successful in preventing further reflux

Meeting Dec 7 1970

Christmas meeting with talk by the author
Palle Lauring on the conditions under which
children have grown up during the centuries

Combined meeting with the Danish Society
for Clinical Chemistry and Clinical Physiology
Dec 12 1970 on Inborn errors of metabo-
lism

N J Brandt *Laboratory problems in the dia-
gnosis of inborn errors*

E Wamberg *Current and future screening*

*methods for congenital metabolic diseases in
newly born infants*

A Dupont *Screening for diseases involving
oligophrenia in children in institutions*

J Clausen *Specific enzyme defects in glycoli-
pid metabolism in the progressive encephalo-
pathies (lysosome defects)*

Fl Guttler *Hyperphenylalaninaemia*

J Christensen & E Lykkegård Nielsen *Cystic
Fibrosis Incidence and screening*

Extraordinary meeting Dec 11, 1970

Sverre Halvorsen (Oslo) *Regulation of ery-
thropoiesis in newly born infants by means of
stimulators and inhibitors*

N J Brandt

Th. Koos & M H Miller *Intracranial tumours of infants and children* Georg Thieme Verlag Stuttgart 1971 415 pp illus DM 130—

The monograph presents a survey of current knowledge in the field of intracranial tumours in infants and children based on 700 brain tumour cases selected from the tumour collection of the Neurosurgical Clinic of University of Vienna. The various conceptions of the authors have been influenced by personal experiences gathered in many years of clinical work at various neurosurgical centres both in the United States and in Europe.

The book is divided into four main sections: the first giving extensive statistical data and devoted to a detailed discussion of increased intracranial pressure and its various manifestations in the age groups concerned. The histological classification of brain tumours, and tumours of neuroectodermal origin in particular is a difficult task and has been subjected to differences in opinion. The authors have followed the classification of K. J. Zulch based on studies of 6000 tumours. The pathologist might find the photomicrographic material chosen as illustrating variations in the histologic appearance of tumours somewhat scarce and insufficient but the monograph is apparently not basically meant for the neuropathologist. The chapter dealing with increased intracranial pressure is well written and illustrated by excellent drawings schematically presenting possible variations in internal pressure at different sites.

Parts II and III deal with general clinical diagnostic and therapeutic problems related to individual brain regions and with the special tumour types grouped by their topography. It is quite obvious that special emphasis has been placed on diagnostic therapeutic methods modified and adapted to suit the particular conditions encountered in children of different ages, deviating from the techniques employed in adults. The survey is brought up to date in every respect. The usefulness of such diagnostic aids as ultrasonography and radio-isotope scan is discussed and radiologic studies employing different contrast materials is beautifully illustrated with different types and locations of tumours. The use of micro-neurosurgical techniques in dealing with parasellar lesions is advocated. In general the authors' notion on surgical and postoperative treatment agrees entirely with current trends in major neurosurgical centres all over the world. The chapter on craniopharyngeomas to a large

part reviews the experiences by Donald B. Matson and co-workers published in 1969.

The last part of the monograph is devoted to a description of chemotherapy of malignant tumours of the central nervous system and also includes a number of suitable references. The authors do not state to what purpose their study was written but it seems to be suitable as supplementary reading in neurosurgery and should be included in the libraries of teaching institutions of pediatrics and neurosurgery.

Lars Granholm

K. Decker & H. Backmund *Pädiatrische Neuroradiologie* Georg Thieme Verlag Stuttgart 1970 193 pp illus DM 79—

This book on neuroradiology in infancy and childhood is focused on the technical and diagnostic problems specific of paediatric disease of the central nervous system and it presupposes somewhat more than elementary knowledge in general neuroradiology. The special procedures discussed include radio-isotope brain scanning as well as cerebral angiography, encephalography, ventriculography and myelography. Attention is also given to the preparation of the patient and to general anaesthesia in connexion with these procedures.

A separate chapter deals with the radiologic examination of a large series of human fetuses illustrating the prenatal development of the skull and vertebral column. It includes observations made at ventriculography and angiography of several specimens providing useful information on the anatomy of the brain and its vasculature before birth. This is followed by a description of the postnatal development and the range of normal variation of the central nervous system as reflected by the neuroradiological findings.

The main part of the book is concerned with congenital and acquired neurologic disease including traumatic injury to the brain and spinal cord in early life. The radiologic manifestation of such disease is elucidated by numerous references to paediatric, neurologic and radiologic literature and by the wide personal experience of the authors. Numerous reproductions of radiographs help to throw light on this highly specialized and rapidly growing field of medicine.

Georg Theander

G T Pack & A H Islami (eds) *Tumors of the liver* Recent results in cancer research Vol 26 Springer Verlag Berlin Heidelberg and New York 1970 304 pp illus DM 56—

Like the previous volumes of the series *Recent Results in Cancer Research* this one is of a high standard presenting itself in an elegant outfit. The subject tumors of the liver is covered from different points of view for example the epidemiology of primary carcinoma of the liver the pathologic anatomy of primary hepatic tumors hepatic carcinoma genesis surgical anatomy of the liver radiation therapy of liver tumors surgical treatment of liver tumors transplantation of the liver and end results in the surgical treatment of liver tumors.

It is unavoidable when many authors write the different chapters that these are of varying quality. A survey on liver regeneration is extraordinarily well written by Bengmark Gothenburg. The chapter of pathology of tumors of the liver in infancy and childhood is excellent too but one may wonder why the occurrence of precocious puberty associated with hepatoblastomas is not mentioned at all.

The book is to be recommended to pathologists surgeons (including surgeons of pediatrics) and hepatologists as a thorough and well written work on tumors of the liver.

T Schüdt

Gosta Rooth *Clinical acid-base and electrolyte balance* Studentlitteratur Lund 1970 80 pp Sw Cr 14 30

The present booklet by Gosta Rooth serves as a useful primer on several specific acid-base problems in producing quantitative views on respiratory compensation as well as the concepts of mixed acid-base disturbance and organic acidosis. The subject matter is not treated systematically and preferably the book should be read as a continuation of Rooth's *Introduction to Acid-Base and Electrolyte Balance* first published in 1966.

The presentation is greatly simplified and largely maintained at a pre graduate level. Hence the book should not be considered adequate for ward practice. Having accepted the widely held misconception of acute deviations of blood base excess (BE) in response to primary changes in P_{CO_2} as artefacts Rooth bases all statements concerning changes in blood BE in terms of values computed for a fixed hemoglobin concentration of 50 g/L. This convention is most unfortunate for a number of reasons. Firstly it obscures the notion of BE as a measure of the blood concentration of (titratable) acid and introduces inconsistencies between stated chemical variables of the sample medium. Secondly it invites violation of sound chemical data by application of interpretative corrections (in the present case based on false assumptions regarding the stability of the slope of the so

called *in vivo* CO_2 absorption line). Thirdly the distributional effect on the blood acid-base status is physiologically relevant and should be registered. And finally the use of a BE_{std} in the description of primary non respiratory disturbances is conceptually misleading.

Still this book offers the uninitiated several valuable short cuts to the understanding of clinical acid-base problems. According to the author it is intended for doctors who cannot spend more than 3-5 hours on a new book. To those it may be recommended.

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Jerome L Schulman describes interesting experimental work in mice aiming at the evaluation of live influenza virus vaccine. The importance of monitoring the antigenic drift of neuraminidase proteins and of evaluating vaccines in terms of their capacity to elicit antibodies to neuraminidase as well as to haemagglutinin are stressed. The author emphasizes that research should continue for an effective live influenza virus vaccine.

Suck and Yohn present an informative survey of the evidence that mammalian viruses are mutagenic agents and do induce chromosomal and mitotic irregularities. The authors point out the need for greater attention to the question of whether vaccination with live attenuated viruses might result in any significant increase of genetically abnormal cells or not.

The majority of the contributions deal with basic virology at the level of viral nuclear acids. The rapid progress of knowledge including methodological innovations and the new concepts of molecular virology make the review of such topics necessary. The book is of particular interest to those interested in these special segments of virology.

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General Index

Authors Index

- Aas E 764
Ackerman D and Flod N E 433
Adam D and Margret W 341
Alexiou D et al 77
Aperia A et al 695
Armata J 471
Ashkenazi A et al 285
- Bartley N 540
Bartsocas C H et al 553
Batstone G F et al 349
Berg K and Celandor O 278
Berg U et al 571
Berg U 669
Bergman L and Isaksson B 630
Byrring Hansen A 571
Boda D et al 90
Bodegård G and Schweder G H 181
Buoni G et al 417
- Casteels-van Daele M and De Gaetano G 203
Celandor O and Berg K 278
Chrysostomoudou C M et al 591
Chunga F and Lardinois R 27
Cocchi P et al 475
- Dahlqvist A and Lindqvist B 488
Damgaard Andersen E et al 559
Dar H et al 479
Donath A 512
Donner M and Torma T 545
- Edler I and Lundström N R 117
Ehrenpreis T et al 209
Eid E E 39
Eid E H and Illingworth H S 333
Ekholm R and Olm P 565
Eklöf O et al 81
Elián E and Iancu T 353
Engel K and Kuldeberg P 637
- Fernandes J and van de Kamer J H 187
Finnström H 685
Flod H E and Ackerman D 433
Foucard T et al 621
- De Gaetano G and Casteels van Daele M 203
Goldschmidt E and Pällsgaard G 146
Gothic A et al 437
Grotte G et al 59
Gustafson K H and Hagberg B 585
- Haahr J and Sparreohn S 716
Hagberg B and Gustafson K H 585
Halveg A B and Haahr J 720
Halvorsen S and Skjælaaen P 301
Harlund Christensen E and Oter J 709
Henriksson P et al 227
Hjalmarsson O et al 11
van der Horst J L and Wadman S K 594
- Iancu T and Elián E 353
Ivanainen M and Palo J 346
Illingworth R S and Eid E E 333
Isaksson B and Bergman L 630
- Joss E E and Zuppinger K A 678
- van de Kamer J H and Fernandes J 187
Kanawati A A et al 309
Kantzyk D et al 465
Kuldeberg P and Engel K 637
Kuntzel H W et al 1
Kleiter B et al 173
Kouba K et al 482
Kringelbach J and Wennevold A 239
Krohn K and Vapaavuori E K 49
- Lardinois R and Chunga F 27
Laskownicka Z et al 456
Lae S O et al 129
Lindqvist B and Dahlqvist A 488
Lundstedt E et al 78
Lommen E J P et al 642
Lundström N R and Edler I 117
Lyon I C T et al 324
- Margret W and Adam D 341
Matoth Y et al 317
Mendicino M et al 407
- Nordio S et al 441
Nordio S et al 449
Norén I et al 269
Norman A H et al 165
- Olegård R and Svennerholm L 505
Olm P and Ekholm R 505
Olm P 578
- Pällsgaard G and Goldschmidt E 146
Palo J and Ivanainen M 346
Pennock C A et al 299
Persson H and Tunell R 385
- Rapola J and Savilahti E 253
Reid M McC et al 295
Reisner S H et al 357
Redler M A C et al 222
Roberts D F et al 158

ANNOUNCEMENT

VIII MEDITERRANEAN AND MIDDLE EASTERN PAEDIATRIC CONGRESS

This Congress sponsored by the Union of the Mediterranean and Middle Eastern Paediatric Congress and the Spanish Paediatric Society will be held in Barcelona (Spain) from 12th till 15th of March 1972 at the Children's Hospital of the Seguridad Social. President Dr A. Ballabriga.

Three main topics will be discussed

Newborns

Nutritional Problems in Childhood

Cancer and Leukaemia in Childhood

Several round tables and symposia upon other topics are also contemplated.

Official Languages: Spanish, English and French. Simultaneous translation will be provided.

For advance program detailing the planning and organizing of the Congress and providing all symposium and workshop titles, advance registration and hotel reservations forms, write to: Secretariat VIII Mediterranean and Middle Eastern Paediatric Congress, Dr A. Gallart, Clínica Infantil de la Seguridad Social, Paseo Valle de Hebrón s/n, Barcelona, Spain.

Authors Index

- Aas K. 264
 Ackerman D and Flod N ■ 433
 Adam D and Margret W 341
 Alexiou D et al 93
 Aperia A et al 695
 Armata J 471
 Ashkenazi A et al 785

 Barclay N 540
 Bartsocas C S et al 553
 Batstone G F et al 349
 Berg K. and Celander O 278
 Berg U et al 571
 Berg, U 669
 Bergman L and Isaksson B 630
 Bjerring Hansen A 571
 Boda D et al 90
 Bodegård G and Schwabler G H 181
 Bucci G et al 417

 Castels van Daele M and De Gaetano G 703
 Celander O and Berg K 278
 Chrysostomidou C M et al 491
 Chunga F and Lardinois R 27
 Cocchi P et al 475

 Dahlqvist A and Lindqvist B 488
 Damgaard Andersen E et al 559
 Dar H et al 479
 Donath A 517
 Donner M and Torma T 545

 Edler I and Lundstrom N R 117
 Ehrenpreis T et al 409
 Eid E E 39
 Eid E E and Illingworth R S 333
 Ekholm R and Olin P 565
 Eklof O et al 81
 Elian E and Iancu T 353
 Engel K and Kildeberg B 637

 Fernandes J and van de Kamer J H 187
 Finnstrom O 685
 Flod N E and Ackerman D 433
 Foucard T et al 621

 De Gaetano G and Castels-van Daele M 203
 Goldschmidt L and Pallsgaard G 146
 Gotlieb A et al 437
 Grotte G et al 59
 Gustafson K H and Hagberg, B 585

 Haahr J and Sparrebohm S 216
 Haahr J and Halveg A ■ 720

 Hagberg B and Gustafson K H 585
 Halveg A B and Haahr J 720
 Halvorsen ■ and Skjaelaaen P 301
 Harlund Christensen E and Øster J 709
 Henriksson P et al 227
 Hjalmarsson O et al 11
 van der Horst J L and Wadman S K 594

 Iancu T and Elian E 353
 Ivanainen M and Palo J 346
 Illingworth R S and Eid E E 333
 Isaksson B and Bergman L 630

 Joss E E and Zuppinger K A 678

 van de Kamer J H and Fernandes J 187
 Kanawati A A et al 309
 Karitsky D et al 465
 Kildeberg P and Engel K 637
 Kintzel H W et al 1
 Klettner B et al 173
 Kouba K et al 482
 Kringelbach J and Wennevold A 239
 Krohn K and Vapaavuori E K 49

 Lardinois R. and Chunga F 27
 Laskownicks Z et al 456
 Lie S O et al 129
 Lindqvist B and Dahlqvist A 488
 Lindstedt E et al 78
 Lommen E. J P et al 642
 Lundstrom N R and Edler I 117
 Lyon I C T et al 324

 Margret W and Adam, D 341
 Matoth Y et al 317
 Mendicino M et al 407

 Nordio S et al 441
 Nordio S et al 449
 Norén I et al 269
 Norman A et al 165

 Olegård R and Svennerholm L 505
 Olin P and Ekholm, R. 505
 Olin P 578

 Pallsgaard G and Goldschmidt E 146
 Palo J and Ivanainen M 346
 Pennock C A et al 299
 Persson B and Tunell R 385

 Rapola J and Savilahti, E 253
 Reid M McC et al 295
 Reissner S H et al 357
 Rudler M A C et al 22
 Roberts H F et al 158

- Samánek M et al 149
 Samuelson G 653
 Savilahti E and Rapola J 253
 Say II et al 197
 Schettini F et al 17
 Schuler D et al 66
 Schwarze R et al 705
 Schweisguth O et al 6
 Schwieler G H and Bodegård G 181
 Severi F et al 716
 Skjælaaen P and Halvorsen E 301
 Skrede II et al 138
 Sparrevoth II and Haahr J 216
 Spennati G F et al 192
 Sterky G et al 461
 Svennerholm L and Olegård R 505
 Szczepski O et al 73
 Sovik O et al 428

- Tan K L 329
 Taysi K et al 235
 Tomsovic E J et al 647
 Tondeur M et al 98
 Tunell R and Persson II 385
 Turhan Ö and Özsoylu S 338
 Torma T and Donner M 545

- De Vaan G A M et al 22
 Vahlquist B et al 533
 Vapaavuori E K and Krohn K 49
 Visakorpi J K et al 666
 Vogeli II et al 528
 Wadman E K and van der Horst J L 594
 Wennevold A and Kringelbach J 239

- Zamet P and Chung F 33
 Zuppinger K A and Joss II E 678

- Öhman R et al 399
 Øster J and Harlund Christensen E 709
 Özsoylu S and Turhan Ö 338

Subject Index

- Acidosis**
 influence on lipid mobilization 385
 recurrent urinary tract infections 521
- Alkalosis**
 metabolic in infants role of water depletion 637
- Amino acids**
 branched-chain keto aciduria 594
 cystathioninuria normal infants 324
- Asphyxia**
 residual placental blood volume 433
- Asthma**
 house dust allergy hyposensitization 264
- Ataxia telangiectasia** 66
- Bacteriology**
 mycoplasma pneumoniae 726
 neonatal meningitis salmonella species 540

Bilirubin

- effect of orotic acid 1 705
 neonatal icterus glucuronic acid excretion 437
 neonatal icterus HBABA index 105 106
 unbound microdetermination 27 33
 unbound separation by gel filtration 27 33
- Blood**
 coagulation disorder in giant haemangioma 227
 ectopic spleen Rh incompatibility 353
 erythropoiesis inhibition of by plasma from newborns 301
 fibrinogen turnover prematures 465
 leukaemia congenital 720
 leukaemia rubidomycin treatment 471
 leukaemia treatment with L asparaginase 22
 lymphoblastic transformation ataxia telangiectasia 66
 plasma fatty acid composition infants 505
 prothrombin newborns and first year of life 269
 purpura acetylsalicylic acid therapy 203
 red cell phosphoglycerides infants 505
 red cells acid lysis 17
 red cells foetal stability of acid phosphatase 192
 red cells postnatal changes 317
 red cells purine nucleotides in Lesch Nyhans syndrome 642

Cardiovascular system

- acyanotic heart disease radiographic changes 727
 arterial anomalies compression of oesophagus and trachea 81
 cardiac syncope prolonged Q T interval 239
 cardiomegaly neonatal hypoglycaemia 295
 circulatory adaptation in thermoregulation 278
 extrasystolia new malformation syndrome? 559
 placental blood volume asphyxia 433
 pulmonary blood flow cystic fibrosis 149
 ultrasound-cardiography 108 117

Chromosomes

- ataxia telangiectasia 66
 small extra chromosome in Prader Willi syndrome 22
 triploidy live born patient 246
 trisomy 18 incidence in Greece 591
 trisomy 18 ovarian dysgenesis 93
 X₂XXY anomaly 249
 XY/XO gonadal dysgenesis 716

Cutis verticis gyrata 346

- cutis lata growth retardation 357
 Cystathioninuria 324

Dermatoglyphics 479

- Diabetes**
 dental disease and diet 461
 hyperosmolar coma 247
 insula resistant anaphylaxis 647
- Diastrophic dwarfism** 243
Duchenne muscular dystrophy 428

Endocrine organs

- adrenal cholesterol splitting enzyme 611
 adrenal cortical atrophy cerebral sclerosis 61
 adrenal foetal 605
 adrenal β hydroxysteroid dehydrogenase 61:

- carbamazole treatment influence on foetus 565
- corticotropin antibodies to 614
- cortisol binding proteins determination of 617
- cortisol competitive protein binding 618
- Cushing's syndrome pituitary tumor following 609
- C₁₇-steroids difficulties by determination 616
- dehydroepiandrosterone excretion in adrenal disorders 616
- dehydroepiandrosterone excretion in cystic fibrosis 617
- gonadal dysgenesis XY/XO mosaicism 716
- growth factor deficiency 610
- growth hormone glucose tolerance and insulin secretion 678
- growth hormone isolated deficiency 607
- growth hormone normal adolescents 606
- growth hormone prenatally deficiency 607
- hypoadosteronism 614 726
- hypogonadism 608
- ovarian dysgenesis trisomy 18 183
- pituitary dwarfism high serum HGI 606
- pseudohermaphroditism 17 hydroxylase deficiency 612
- puberty delayed evaluation of pituitary function 610
- puberty excretion of pregnanediol and triol 615
- puberty precocious hamartoma of median eminence 610
- puberty precocious treatment with chlormadinone 611
- puberty precocious treatment with medroxyprogesterone 611
- steroid metabolism, newborns 605
- steroids identification in urine 618
- testes foetal 604
- testosterone binding to plasma proteins 616
- testosterone determination in plasma 615
- thyroid fetal 613
- thyroid proteins childhood goitre 578
- thyrotropin, clearance rate 608
- Turner's syndrome calcium metabolism in 73
- 11 β hydroxylase deficiency 613
- 17 ketosteroids gas liquid chromatography 615
- Enuresis 243 744
- Enzymes
 - alkaline phosphatase duodenal juice rickets 338
 - coenzyme Q Duchenne muscular dystrophy 428
- Epididymitis 216
- Foetus
 - adrenal 605
 - asphyxia placental blood volume 433
 - carbamazole treatment early pregnancy 565
 - growth hormone deficiency 607
 - red blood cells stability of acid phosphatase 192
 - testes 604
 - thyroid influence of propylthiouracil 603
 - thyroid maturation 603
- Gastrointestinal tract
 - abdominal pain recurrent 724
 - absorption bile salt metabolism 377-381
 - alkaline phosphatase duodenal juice 338
 - amino acids faecal ultrafiltrate 373
 - arylamidase brush border membrane 366
 - cow's milk intolerance malabsorption 372
 - Crohn's disease 104 105 209 249 373-376
 - cystic fibrosis pulmonary blood flow 149
 - cystic fibrosis vitamin A deficiency 371
 - disaccharidase deficiency diagnosis of 187
 - enterokinase localization of 369
 - fructose intolerance a glucosidase 364
 - gastroenteritis treatment with antibiotics 110
 - gastric emptying infants 105 370
 - glucose and amino acid absorption 371
 - intestinal biopsy IgA deficiency 363
 - lactic acid influence on absorption and metabolism 367
 - lactose intolerance malnutrition 488
 - lactose malabsorption schoolchildren 365
 - lipid pattern hepatitis
 - liver metabolism in mongolism 372
 - oesophagus compression by arterial anomalies 111
 - oesophagus reconstruction of 59
 - pancreatic proteinases 369
 - pancreozymin secretin test 370
 - protein losing enteropathy 371
 - regional enterocolitis 104 105 209 249 373-376
 - resection of small bowel chronic diarrhoea 366
 - rose bengal test 373
 - small intestine in cystic fibrosis 367
 - starch digestion of in sucrase isomaltase deficiency 364
 - ulcerative colitis azathioprine treatment 376
 - ultrastructural changes after gluten 363
 - xylose-disaccharidase tolerance test 187
 - β galactosidases 364
- Glycosaminoglycan excretion newborns 299
- Growth
 - failure to thrive in Lebanon 309
 - following failure to thrive 39
 - head circumference infants 333
 - hypogonadism 608
 - infants relation to gestational age 685
 - intrauterine retardation cutis laxa 357
 - Prader test and plasma HGH 108
 - standards for height 608
- Head circumference 333 685
- Hemispherectomy 545
- Hypoglycaemia
 - cardiomegaly newborns 295
- Immunology
 - anaphylaxis insulin resistant diabetes 647
 - ataxia telangiectasia delayed type skin reaction 66
 - coproantibodies to milk proteins 173
 - immunofluorescence congenital nephrotic syndrome 253
 - IgE levels in asthmatic bronchitis 621
- Infants
 - alkalosis metabolic in water depletion 637
 - development in nursery homes v private families 571
 - gastric emptying 105

- Samánek M et al 149
 Samuelson G 653
 Savilahti E and Rapola J 253
 Say B et al 197
 Schettini F et al 17
 Schuler D et al 66
 Schwarze R et al 705
 Schweisguth O et al 6
 Schwieler G H and Bodegård G 181
 Severi F et al 716
 Skjælaaen P and Halvorsen S 301
 Skrede S et al 138
 Sparrevoth S and Haahr J 216
 Spannati G F et al 192
 Sterky G et al 461
 Svennerholm L and Olegård R 505
 Szczepski O et al 73
 Sovik O et al 428

- Tan K L 329
 Taysi K et al 235
 Tomsovic E J et al 647
 Tondeur M et al 98
 Tunell R and Persson B 385
 Turhan Ö and Özsoylu S 338
 Tormä T and Donner M 545

- De Vaan G A M et al 22
 Vahlquist B et al 533
 Vapaavuori E K and Krohn K 49
 Visakorpi J K et al 666
 Vogeli B et al 528
 Wadman S K and van der Horst J L 594
 Wennevold A and Kringelbach J 239

- Zamet P and Chunga F 33
 Zuppinger K A and Joss E E 678

- Öhman R et al 399
 Öster J and Harlund Christensen E 709
 Özsoylu S and Turhan Ö 338

Subject Index

Acidosis

- influence on lipid mobilization 385
 recurrent urinary tract infections 521

Alkalosis

- metabolic in infants role of water depletion 637

Amino acids

- branched-chain keto aciduria 594
 cystathioninuria normal infants 324

Asphyxia

- residual placental blood volume 433

Asthma

- house dust allergy hyposensitization 264

Ataxia telangiectasia 66

Bacteriology

- mycoplasma pneumoniae 726
 neonatal meningitis salmonella species 540

Bilirubin

- effect of orotic acid 1 705
 neonatal icterus glucuronic acid excretion 437
 neonatal icterus HBABA index 105 106
 unbound microdetermination 27 33
 unbound separation by gel filtration 27 33
Blood
 coagulation disorder in giant haemangioma 227
 ectopic spleen Rh incompatibility 353
 erythropoiesis inhibition of by plasma from newborn 301
 fibrinogen turnover prematures 465
 leukaemia congenital 720
 leukaemia rubidomycin treatment 471
 leukaemia treatment with L asparaginase 22
 lymphoblastic transformation ataxia telangiectasia 6
 plasma fatty acid composition infants 505
 prothrombin newborns and first year of life 269
 purpura acetylsalicylic acid therapy 203
 red cell phosphoglycerides infants 505
 red cells acid lysis 17
 red cells foetal stability of acid phosphatase 192
 red cells postnatal changes 317
 red cells purine nucleotides in Lesch Nyhans syndrome 642

Cardiovascular system

- acyanotic heart disease radioeraphic changes 727
 arterial anomalies compression of oesophagus and trachea 81
 cardiac syncope prolonged Q T interval 239
 cardiomegaly neonatal hypoglycaemia 295
 circulatory adaptation in thermoregulation 278
 extrasystolia new malformation syndrome? 559
 placental blood volume asphyxia 433
 pulmonary blood flow cystic fibrosis 149
 ultrasoundcardiography 108 117

Chromosomes

- ataxia telangiectasia 66
 small extra chromosome in Prader Willi syndrome 22
 triploidy live born patient 246
 trisomy 18 incidence in Greece 591
 trisomy 18 ovarian dysgenesis 93
 XYY anomaly 249
 XY/O gonadal dysgenesis 716

Cutis verticis gyrata 346

Cutis laxa growth retardation 357

Cystathioninuria 324

Dermatoglyphics 479

Diabetes

- dental disease and diet 461
 hyperosmolar coma 247
 insulin resistant anaphylaxis 647

Diastrophic dwarfism 243

Duchenne muscular dystrophy 428

Endocrine organs

- adrenal cholesterol splitting enzyme 611
 adrenal cortical atrophy cerebral sclerosis 613
 adrenal foetal 605
 adrenal β hydroxysteroid dehydrogenase 612

serum bilirubin 1
therapy controlled trial 407 417
Psychiatry
consultations in paediatrics 112
psychotherapy 112
Purpura acetylsalicylic acid therapy 203

Respiratory tract

asthmatoïd bronchitis IgE levels in 621
control of respiration in newborns 181
hyaline membrane disease peritoneal dialysis in 90
IRDS fibrinogen turnover 465
IRDS follow up study 102
mycoplasma pneumoniae infections 726
pulmonary blood flow cystic fibrosis 149
trachea compression by arterial anomalies 81
Rubidomycin 471

Sarcoidosis hypercalcaemia renal function 249

Suman cressé 479

Social paediatrics

child health in northern Sweden 653
failure to thrive in Lebanon 309
menarche physique in industrial area 158
nursery home v private families 571

Spleen

ectopic Rh incompatibility 353

Surgery

anorectal 495-497
cardiovascular 497-499
general 499-502
Synchua vulvae 709

Tay Sachs disease 399

Third fontanelle 329

Toxoplasmosis

hepatic involvement 482

Trisomy 18 93

Tuberosc sclerosis newborns 349

Tumor

giant haemangioma 227
mastocytoma 109
thecoma non functioning 6
Wilm's tumor 109

Turner's syndrome calcium phosphate metabolism in 7

Urology

epididymitis in children 216 248
malformations urinary infections 730
synchua vulvae 709
vesico-ureteric reflux 730

Virology

serology in asthmatoïd bronchitis 621
Ward's syndrome 239 248

- maturity studies 685
- plasma fatty acids influence of diet on 505
- red cell phosphoglycerides, influence of diet on 505
- Infections
 - mycoplasma pneumoniae 726
 - nitroblue tetrazolium reduction 475
 - salmonella meningitis 540
 - toxoplasmosis hepatic involvement 482
- Krabbe's disease 103
- L-asparaginase 22
- Lactic acidosis fatal congenital 129
- Lesch-Nyhan syndrome 642
- Lipodystrophy 602
- Liver
 - giant cell transformation Niemann-Pick disease 285
 - hepatitis serum lipid pattern 368
 - metabolism in mongolism 372
 - toxoplasmosis acquired 482
- Lowe's syndrome 146
- Malabsorption
 - test meal in 165
- Malformations
 - developmental anomalies new syndrome? 559
 - diastrophic dwarfism 243
 - dysmorphogenesis a new syndrome 197
 - imperforate anus and skeletal malformations 197
 - oculo-cerebro-renal syndrome 146
 - oculodentodigital dysplasia syndrome 235
 - triploidy live born patient 246
- Malnutrition
 - cerebral ventricles 533
 - lactose intolerance 488
- Maple syrup urine disease 103 594
- Menarche age at in industrial area 158
- Metabolism
 - alkalosis water depletion 637
 - calcium fractions in plasma 630
 - generalized lipodystrophy fat and carbohydrate metabolism 602
 - growth hormone glucose tolerance and insulin secretion 678
 - hypomagnesemia hypocalcemia 441 449
 - lactic acidosis fatal congenital 129
 - leucinoses atypical form 103
 - lipid mobilization newborn infants 385
 - plasma fatty acids influence of diet on 505
 - red cell phosphoglycerides influence of diet on 505
 - sodium homeostasis urinary tract infections 695
 - Turner's syndrome calcium phosphate metabolism in 73
- Metachromatic leucodystrophy 585
- Methodology
 - bilirubin unbound determination of 27 33
 - glomerular filtration rate renal plasma flow 512
 - haemodialysis arteriovenous fistula for 78
 - HBABA index 104
 - thyroxine microanalysis of 246
 - ultrasoundcardiography 108 117
 - Uricult® 245

- xylose-disaccharide tolerance test 187
- Mongolism
 - liver metabolism 372
- Mucopolysaccharidosis
 - glycosaminoglycan excretion 299
 - infant 98
- Mycology
 - new antifungal substance 341
 - pimaricin treatment 456

Nephrology

- acid base balance defective regulation in infections 52
- glomerular filtration rate 512 528
- haemodialysis arteriovenous fistula for 78
- nephrotic syndrome congenital immunofluorescence 263
- recurrent infections acid base balance 521
- renal function tests urinary tract infection 669
- renal plasma flow 512 528
- sodium homeostasis urinary tract infection 695
- Nervous system
 - cerebral hypoxia EEG in 110
 - cerebral ventricles size of in malnutrition 533
 - Duchenne muscular dystrophy coenzyme Q 428
 - hemispherectomy hemiplegia epilepsy 545
 - infantile globoid cell leucodystrophy 103
 - infantile spasms recovery from 729
 - meningitis neonatal salmonella 540
 - metachromatic leucodystrophy incidence of 585
 - muscular weakness intermittent in malformation syndrome 559
 - reflex epilepsy 109
- Newborn infants
 - cardiomegaly neonatal hypoglycaemia 285
 - circulatory adaptation thermoregulation 278
 - control of respiration 181
 - dermatoglyphics 479
 - diuretics effect when given during pregnancy 727
 - erythropoietic inhibitors 301
 - glycosaminoglycan excretion 299
 - lipid mobilization 385
 - nitroblue tetrazolium test 475
 - prothrombin 269
 - salmonella meningitis 540
 - steroid metabolism influence of thyroid 605
- Niemann-Pick disease 285
- Nitroblue tetrazolium test 475

- Orotic acid serum bilirubin 1 705

Phenylketonuria

- incidence in Finland 666
- mild and severe forms 11

Poisoning

- lead accumulation treatment of 553

Prader-Willi syndrome 222

Premature infants

- circulatory adaptation thermoregulation 278
- fibrinogen turnover 465
- intensive care 49
- intravenous administration of fat 102
- peritoneal dialysis in IRDS 90

serum bilirubin 1
therapy controlled trial 407 417
Psychiatry
consultations in paediatrics 112
psychotherapy 112
Purpura acetylsalicylic acid therapy 203

Respiratory tract
asthmatoïd bronchitis, IgE levels in 621
control of respiration in newborns 181
hvaline membrane disease peritoneal dialysis in 90
IRDS fibrinogen turnover 465
IRDS follow up study 102
mycoplasma pneumoniae infections 746
pulmonary blood flow cystic fibrosis 149
trachea compression by arterial anomalies 81
Rubidomycin 471

Sarcoidosis hypercalcemia renal function 249
Sunscreen 479
Social paediatrics
child health in northern Sweden 653
failure to thrive in Lebanon 309
menarche physique in industrial area 158
nursery home v private families 571
Spleen
ectopic Rh incompatibility 353

Surgery
anorectal 495-497
cardiovascular 497-499
general 499-502
Synchia vulvae 709

Tay Sachs disease 399
Third fontanelle 329
Toxoplasmosis
hepatic involvement 482
Trisomy 18 93
Tuberose sclerosis newborns 349
Tumor
giant haemangioma 227
mastocytoma 109
thecoma non functioning 6
Wilm's tumor 109
Turner's syndrome calcium phosphate metabolism in 7

Urology
epididymitis in children 216 248
malformations urinary infections 730
synchia vulvae 709
vesico-ureteric reflux 740

Virology
serology in asthmatoïd bronchitis 621
Ward's syndrome 239 248